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Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites

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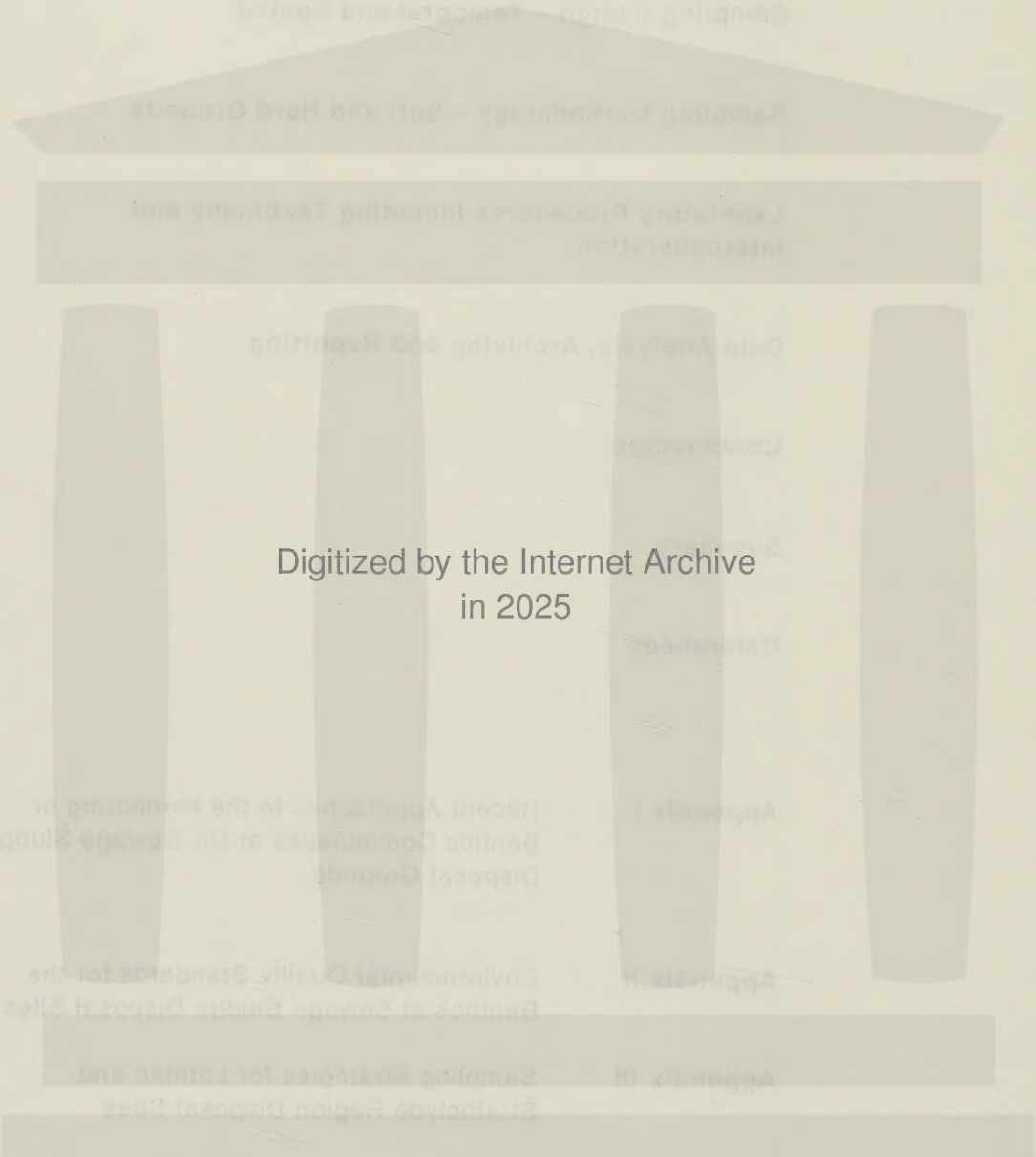

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Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites

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***Now at Scottish Environmental Advisory Services**

(Prepared by the Biology Task Team for the MPMMG Coordinating Group on Monitoring of Sewage Sludge Disposal Sites)

Preface

Statutory control of the dumping of sewage sludge to sea in United Kingdom waters dates back to 1974, with implementation of the Dumping at Sea Act (1974) (DASA). Since then, the requirement to monitor the effectiveness of licence conditions through field assessment of effects has been met in England, Wales and Northern Ireland by the statutory authorities, Ministry of Agriculture, Fisheries and Food (MAFF) and Department of Environment (Northern Ireland) (DOE(NI)), though some of the larger disposal operations have received additional attention, either by licensees in consultation with the statutory authorities, or by research workers with a general interest in the responses of marine systems to waste discharges. However, in 1979 the licensing authority in Scotland, Department of Agriculture and Fisheries for Scotland (DAFS), introduced the concept of self-monitoring whereby licensees at selected disposal sites would undertake an agreed component of the monitoring activity – usually with the emphasis on spatial studies – as a formal requirement of the dumping licence. Following implementation of Part II of the Food and Environment Protection Act (1985), which superseded DASA, and in recognition of an enhanced need for monitoring a variety of wastes which are disposed of by ships to sea, "self-monitoring" was introduced in England, Wales and Northern Ireland.

Accompanying this development, the UK Marine Pollution Monitoring Management Group (MPMMG), a non-statutory body comprising experts drawn from government, research and water industry establishments, has recognised the requirement for improved harmonisation in the monitoring of sewage sludge disposal grounds, so as to allow for UK–wide assessments of the effectiveness of ongoing scientific programmes, and generally to enhance the quality of data generated from the increasingly large number of organisations involved. One result of this was the setting up in 1987 of a Coordinating Group on Monitoring of Sewage Sludge Disposal Sites (CGMSD) to deal with these and related issues. To date, much of the technical input to this Group has been via a series of expert Task Teams.

The following report presents the outcome of work by the Biology Task Team, which during 1988 was charged with reviewing approaches to the field monitoring of the benthic fauna. A complementary summary of this account, along with reports from other Task Teams dealing with sediment chemistry, microbiology and "method-determined parameters", has been prepared by the above Coordinating Group (MPMMG, 1989).

On 5 March 1990 the UK Government announced its intention to stop the disposal of sewage sludge at sea by the end of 1998. Until then such operations may continue under annual licence and will be subject to monitoring including the field monitoring of benthic fauna described in this Report.

Introduction

The benthic macrofauna has been the traditional target for the routine monitoring of biological effects at sewage sludge disposal sites. This is because:

i) sea bed sediments represent the ultimate sink for most sludge-associated contaminants;

ii) most macrofaunal species are long-lived (>1year) and sedentary, and so can provide a measure of contaminant effects integrated over time;

iii) they are sensitive to anthropogenic influences, and their responses are to a large extent predictable;

iv) they are amenable to quantitative sampling;

v) they are well studied scientifically, compared with other sediment-dwelling components (eg. the smaller meio-and microfauna), and taxonomic keys are available for most groups;

vi) there may be direct links with valued resources, eg. fish (via feeding) and edible molluscs.

A notable feature of benthos monitoring programmes to date is the wide variation in sampling and analytical methodology. There are two main reasons why absolute uniformity in the conduct of such programmes cannot be achieved, namely:

i) substratum type, which may range from fine muds to exposed bed rock, requiring very different sampling approaches;

ii) water movements, which may account for differences not only in the prevailing bottom substrata and associated biota but also in the degree and spatial extent of any impact of the waste discharge.

A detailed review of past and current practices is given in Appendix I. This illustrates the large effort expended in benthic community monitoring at the sewage sludge disposal grounds during the past 15 years. Appendix I and Table I with its accompanying footnotes amply illustrate the disparity in approaches. This is, in part, an inevitable consequence of the widely different environmental conditions prevailing at UK sludge disposal sites. Despite this, there is clearly scope for greater harmonisation, and this report makes a series of proposals to this effect.

These proposals are designed to meet the following objectives for disposal site monitoring:

i) to define the intensity and spatial extent of effects on the benthos which are attributable to sludge dumping;

ii) to establish whether there is a worsening trend in intensity or extent of effects, beyond those deemed to be permissible, following stabilisation of the initial response to sludge inputs.

In order to achieve these objectives, it is emphasised that, along with standard methods for biological sampling, concurrent sampling of sediments for the determination of relevant physical and chemical properties is essential for the accurate interpretation of benthic changes. The account begins with a review of requirements for effective sampling design and then deals with the issues of field sampling methodology, sample processing and data analysis. Proposals for standardisation in approaches are made at each stage. The account also makes proposals concerning the setting of environmental quality standards using the benthos; these are given in Appendix II. Here, the importance of an evaluatory phase prior to application is emphasised.

Sampling Design – Temporal and Spatial

The objectives of any monitoring programme are to detect any system disturbance greater than that caused by natural environmental variability and to identify the cause(s) of such disturbance. Careful thought must be given therefore to sampling design prior to the initiation of the survey to allow distinctions to be drawn between natural and anthropogenically induced variability. Both spatial and temporal trends need to be assessed, and thus both the timing and the extent of sampling and the type of environmental and biological data to be collected are important considerations. These factors, together with those arising from local financial and logistical constraints, will govern the design of the programmes. Examples of two sampling designs are given in Appendix III, and a model strategy is shown in Figure 1. The principles and practice underlying good sampling design and data analysis are cogently stated in Green (1979) and McIntyre, Elliott and Ellis (1984).

Figure 1. Model strategy summarising survey design.

Four stages may be identified in planning, initiating and undertaking a survey, *viz* an initial desk study, followed successively by exploratory, baseline and ongoing surveys. Prior knowledge or limited objectives may remove the need for some of these stages, however overall design criteria will be considered progressively for each stage.

Desk Study

An initial consideration of existing information on the types of disturbance expected and on the survey area will allow the overall survey design to be planned.

Distribution of sampling stations

This will be influenced by the following:

i) Type of disturbance anticipated. For example, assessment of point source pollution is best done by trend analysis of data from a grid or transect(s) of sampling stations. The influence of generally disseminated contaminants is better understood from comparative analyses of data from stations randomly distributed over the survey area (but see qualifications below to random selection of stations).

ii) The local topography and hydrography. For example, a uniform depth and substratum type over the sampling area will allow the establishment of a regular sampling pattern constrained only by consideration of the anticipated disturbances. Tidal and residual currents must be considered in the survey design. Irregular depths and variable substrata in the area will necessitate the imposition of some form of stratification to whatever sampling pattern has been chosen as suitable to the type of disturbance to be assessed.

Number of samples taken at each sampling site

This will be influenced by:

i) The need for statistical accuracy in assessing sample heterogeneity. At least 10 samples are usually required to obtain a fully representative array of the commonly occurring species together with some measure of their variability.

ii) Operational constraints. These factors interact in dictating a practical compromise for any particular survey.

iii) Site characteristics. Local complexities and heterogeneities will influence the number of samples necessary to establish community variability, ie. from the outset the design of any survey will necessarily be site specific.

For most practical purposes a single sample taken at each of as wide as possible a number of stations will be adequate to assess the general distribution of communities over a survey area. If quantification of the fauna is required then a minimum of three samples should be taken at each site (for further details see pages 7 and 8).

Frequency of sampling

This will be dictated by individual survey requirements. Spatial patterns may be assessed from data obtained on a single sampling occasion, but assessment of temporal trends can be made only from repeated sampling. Obtaining information on seasonal changes may require a sampling frequency related to the life cycles of the principal organisms present in the area (see below). Moreover, fluctuating input levels of the agent(s) responsible for change may impose a sampling time scale.

Faunal components to be measured

This is also survey dependent, but certain generalisations may be made. The macroinfauna are advocated as the target component of the fauna. Their size range (conventionally 0.5—10 mm) enhances collection, extraction and identification and the wealth of background and comparative data available on this group of organisms greatly increases their usefulness. Moreover, the comparative immobility and longevity of their mature stages within the sediments allows their populations to be viewed as integrators of sedimentary environmental impacts over long periods.

Other faunal components frequently advocated for either special or general surveys include meiofauna (conventionally 63 μm—0.5 mm), epifauna and demersal fish. Meiofauna have generally shorter life cycles than macrofauna, making them less suitable for the study of long term changes. However, they are known to respond similarly to the macrofauna in disturbed situations and may be suitable target organisms in particular areas. Sessile epifauna may be a suitable target in some areas. Motile epifauna and fish may avoid contaminated or disturbed areas and this reduces their general suitability as targets. Owing to their greater size and the commercial value of some of these species, however, their inclusion in surveys can be recommended.

Environmental components to be measured

The measurement of certain basic environmental variables at each sampling station is essential to a proper interpretation of biotic change. These should include water depth, sediment particle size distribution, organic content and redox profiles (0—10 cm). Measurement of chemical contaminants and/or disturbance parameters and the recording of the presence of sewage artefacts is essential and will be survey dependent. It is usually feasible to assess these at more stations than are sampled for biological communities.

Exploratory Survey

Having designed the probable form of the required survey in the desk study, an exploratory survey of the study area is required in order to define those topographic and environmental details necessary for a specific survey plan.

Choice of techniques

Rapid qualitative or semi–quantitative techniques may be used in an exploratory survey of an area rather than the fully quantitative techniques used in baseline and ongoing surveys. Thus visual assessment of the distribution of substrate types and communities over an area can be made on board ship from a succession of sediment samples (taken by any appropriate means) and/or by using underwater video and photography. The recently developed REMOTS technique (Rhoads and Germano, 1982), which combines sediment profiling with photography, may be particularly suitable for this type of preliminary synoptic survey. Such sedimentary explorations should be combined with trawl samples for epifauna and fish.

Extent of survey

The exploratory survey should cover the entire area of actual or potential disturbance and in addition must include a comparable reference area outside the influence of the potential effects on the benthos.

Intensity of survey

All major habitat types within the survey area should be explored and mapped, at least in outline, and a visual estimate of the distribution of the immediately obvious infaunal and epifaunal organisms present in each habitat obtained.

Baseline Survey

The results of the exploratory survey should be used to design the details of a baseline survey which will establish the distribution and abundance of the target fauna throughout the area and record the appropriate environmental data.

Choice of techniques

Quantitative techniques should be used where possible. Sediment samples of known volume should be obtained using grabs and/or corers. Different techniques will be needed on hard substrata (see section on Sampling Methodology). Beam trawl samples should also be taken.

Extent of sampling area

The exploratory survey data should be used to tailor the baseline survey area to the outer limits of the area seen to be threatened by disturbance. An additional reference area must be sampled which should include appropriately representative habitats.

Distribution of sampling stations

If the fauna and substrata are relatively uniform throughout the area then no additional criteria to those listed in the desk study need be considered. The presence of variable substrata and communities will necessitate some degree of stratification in the sampling regime. Enough sampling stations should be included to allow an adequate description of each habitat type within the area of interest. Ideally stations should be randomly distributed within each habitat type ("stratified random sampling") and allocated in proportion to the size of the habitat (Green, 1979). However, if a choice has been made to institute a grid or transect survey then randomisation is inappropriate, but sufficient grid or transect points should be included to place a minimum of two into each habitat type.

Number of samples taken

To establish the distribution of communities over the area it may be sufficient to take a single sample at as many sampling points as practicable, given the financial and time constraints of any particular survey. Should quantification of populations be a requirement of the baseline survey, then a minimum of three replicates should be taken at each of a selected number of stations considered to be representative of the major communities present in the area. This will provide adequate quantification of the commoner species present but will not quantify the less common or establish the full range of rarer species. For example, in soft silt, three samples might be expected to collect 60% of the species present in an area, and five samples would usually yield over 70%. The latter number should be preferred in quantitative surveys at least at those stations likely to be chosen for later repetitive sampling in the ongoing survey (see below). For further discussion of these species/area relationships see Swartz (1978), Green (1979),

McIntyre *et al.*, (1984) and Codling and ap Rheinallt (1987) and for techniques for achieving appropriate levels of precision, see Holme and McIntyre (1984).

Frequency of sampling

Baseline surveys frequently rely on data taken on a single sampling occasion. If this is so then the timing of the sampling period should be carefully considered. All subsequent repeat or ongoing surveys must be undertaken in the same season to minimise comparative seasonal variability. Ideally baseline survey sampling will be continued at intervals throughout at least one full seasonal cycle to establish the limits of seasonal variability within the survey area.

Faunal components to be measured

The target groups will have been identified in the desk study, and the choice qualified by information obtained in the exploratory survey. For most purposes quantitative data on macrofauna together with semi-quantitative (trawl based) data on epifauna are considered adequate. Ideally, if resources are available, all major faunal levels should be assessed in the baseline survey (ie. micro-, meio-, macro- and epifauna and demersal fish).

Environmental components to be measured

These were identified in the desk study.

Ongoing Survey

The discernment of temporal change requires regular repetitive sampling.

Choice of techniques

These must be identical to those used in the baseline survey.

Extent of survey area and distribution of stations

A baseline survey should be repeated at infrequent intervals, to check the representativeness of regular monitoring sites (see below), and to ensure that no unexpected effects are occurring elsewhere. Regular sampling should be continued at a selected series of stations which should represent a gradient from potentially fully disturbed to potentially undisturbed conditions in at least the dominant habitat type in the area.

Number of samples to be taken

As stated for the baseline survey, the minimum acceptable number of samples to provide statistical analysis of the populations of the most common species at any station is three. If acceptable estimates of the less common species are also required then at least five samples should be taken (McIntyre *et al.*, 1984).

Frequency of sampling

Ideally seasonal changes should be assessed, which would require a minimum of four sampling occasions per year for macrofauna. More often a single annual set of samples is taken, and so sampling must be undertaken in the same season of each year.

Faunal components to be measured

Quantitative sampling of only the major target group(s) should be carried out (see above).

Environmental components to be measured

These are as in the baseline survey.

Sampling Methodology – Soft and Hard Grounds

Any attempt to establish a common sampling strategy for the UK sewage sludge disposal grounds must take into account variations in site characteristics. Broadly speaking, disposal sites range from very low to high degrees of dispersal. Related to this are variations in the nature of the sea bed, ranging from soft muds to areas of hard rock.

To date, the mainstay of most monitoring programmes has been the collection and analysis of grab samples in order to assess benthic infaunal community structure. This is usually coupled with investigations into the physical structure of sediments and their contamination by faecal bacteria and heavy metals. As noted in Appendix I, the techniques used in such studies have varied considerably, with differences in sampling gear, sample numbers, sieve size used for the extraction of fauna, data handling and so on.

The establishment of standard sampling procedures is thus an important step in the process of harmonising approaches to dump site monitoring. For convenience, the following account is divided into approaches to sampling soft and hard substrata, though classification of disposal sites according to their degree of dispersion may eventually prove to be more appropriate.

Soft Substrata

For this purpose, soft substrata are defined as those which are amenable to efficient grab sampling. Many disposal sites will have a range of sediment types within the area to be sampled. Therefore, the proposals outlined here represent a compromise on the ideal procedures for each sediment type.

The use of diver-operated air-lift devices is probably the most efficient way of sampling many soft substrata because they remove the uncertainty associated with operation of remote samplers. However, the operational costs of a fully equipped diving team are high and beyond the reach of many organisations. Additionally, the depth of water, local current speeds and turbidity at most UK disposal sites preclude this use of divers for safety reasons. The use of remotely-operated box corers cannot be recommended for routine monitoring because they need a large vessel and relatively calm weather.

Benthic infauna

The use of grab samplers is recommended for the routine sampling of the infauna of soft sediments. The most commonly used are the van Veen, Smith-McIntyre and Day grabs. Numerous studies have been reported in the literature on the relative efficiency of a variety of grabs, although definitions of efficiency have themselves varied. For a review, see Eleftheriou and Holme (1984).

The Day grab is recommended for routine sampling of sewage sludge disposal sites. It has the advantage over the related Smith-McIntyre grab in that its jaws are not spring loaded, a feature which can pose difficulties in handling in heavy sea conditions. On very fine muds it may prove necessary to use the lighter van Veen grab to prevent the grab burying itself in the sediments.

Most evidence suggests that the bulk of the benthic infauna in terms of numbers lies within the top 5—10 cm of sediment, within the biting distance of most grabs. The following basic procedures should be followed when undertaking grab sampling:

i) The top of the grab should have lifting flaps to allow access to the surface of the sample for collecting subsamples for meiofauna, bacteriology etc.

ii) The grab should sample an area of 0.1 m^2.

iii) The jaws of the grab should shut tight so as to prevent loss of fine material when the grab is recovered from the sea bed (this should be tested before use).

iv) There should be a facility to add weights, as necessary, to improve penetration.

v) On retrieval, an estimate of sample volume should be made, routinely noted, and quantities less than 5 l (7 cm sample depth) in muds and 2.5 l (5 cm sample depth) in hard packed sands should be discarded.

vi) Notes should be made on surface features, texture, colour and smell.

vii) In most circumstances, sub-sampling for other analyses from a grab taken for benthic faunal analysis should not be allowed.

Sample treatment

It has been standard practice in most benthic surveys to consider the macrofauna as the element of the fauna retained on a 1 mm or 0.5 mm mesh screen. This element is then preserved for subsequent sorting, identification and counting. Opinion varies as to the optimum choice of mesh size, and there has been an approximately 50:50 split between 1 mm and 0.5 mm sieves in UK sludge disposal monitoring work.

The use of a 1 mm sieve mesh can be recommended for wide-scale spatial surveys. Small changes in the timing of annual sampling may be reflected in the numbers of juveniles taken, because they undergo high mortality immediately after settlement. This source of variability in abundance estimates will, therefore, be reduced by the use of a 1 mm mesh. At stations selected for temporal trend analysis, samples should be sieved through a 0.5 mm or 1 mm mesh, depending on which retains the bulk of the adult macrofauna. This will inevitably be substratum as well as site specific, though some practical compromise, ie. use of a single mesh size, is invariably necessary when covering a variety of substrata in the same survey.

The following procedures can be recommended for the shipboard handling of samples:

i) Sieving should be as gentle as possible to minimise specimen damage.

ii) The sample should be deposited from the grab into a suitable hopper.

iii) Sieving will be enhanced by gentle "puddling" of the sample with sea water in the hopper prior to sieving.

iv) Suitable instruments (eg. forceps) should be to hand for removing delicate or enmeshed specimens from the sieve mesh.

v) Samples should be fixed in buffered 10% formalin (4% formaldehyde) in sea water (Lincoln and Sheals, 1979) prior to sorting in the laboratory. At this stage vital stains should be applied (see p.13).

Benthic epifauna

Valuable information can be gained from studies of the epifauna, although fully quantitative data can be difficult to obtain. Sampling is usually by various trawls or dredges. Towed underwater video and still photographic equipment (Chapman, 1985; Holme and McIntyre, 1984) can be used effectively for surveying the epibenthos but their use as a scientific tool in disposal site monitoring merits further evaluation. While no one set of equipment is universally applicable to all grounds, the following general recommendations can be made:

i) Either a small 2 m Lowestoft beam trawl (Riley, Symonds and Woolner, 1986) or an Agassiz trawl (Eleftheriou and Holme, 1984) should be used.

ii) The trawl cod-end should have a mesh size not greater than 10 mm (knot to knot).

iii) A minimum of three repetitive tows should be taken at regularly monitored sites.

iv) The distance covered on each haul should be measured by noting the ship's electronic navigator readings at the start and finish of each haul and, in most marine conditions, a towing distance of not less than 500 m should be employed.

v) The catch composition (species and abundance of all organisms, length range and morphological disorders of fish taken) should be recorded and specimens should be retained for further study as necessary.

vi) These trawls should be used to assess the presence of sewage debris on the sea bed and to provide fish and epifauna for chemical and histo–/patho–/micro–biological analyses as required. If insufficient fish are collected by these trawls then larger sized gear, eg. otter trawl, must be used.

vii) As a supplement to trawl sampling, quantitative data can be obtained from underwater television surveys by counting the numbers of prominent species against time/distance travelled, or by the use of still photography to "freeze" the action at regular intervals to provide a transect of sample points along the path of the haul.

It is recognised that further work towards standardisation of epifauna sampling methods is required.

Hard Ground

Hard ground is defined for this purpose as a substratum which is not amenable to quantitative sampling by grab; this applies to several UK disposal sites. In general, quantitative sampling of hard ground will require greater ingenuity than sampling of soft substrata. Direct observation and sample collection by divers, coupled with underwater photography, is probably the most effective way of surveying such areas. However, there are limitations to the routine use of divers, as noted previously.

This is also an area requiring further evaluation of appropriate methods, in particular into improved quantification of community level attributes and the identification of target species for monitoring the condition of the biota of hard grounds.

Owing to the nature of such substrata, the bulk of the fauna will often consist of epibenthic species and the above trawls will be suitable. However, the equipment for sampling stony or rocky grounds will necessarily have to be more robust that that used in areas of softer substrata. The most suitable devices for remote sampling of hard ground (other than exposed bed–rock) are dredges. For example, the naturalist's dredge acts by scraping along the surface of the sea bed and is towed for a fixed time or distance, and the anchor dredge is designed to dig deep into the substratum at one location. Eleftheriou and Holme (1984) give details of design and operation of suitable sampling gear.

Towed underwater television and still photography may be used to good effect on hard ground. However, there is an attendant risk of damage to, or loss of, expensive equipment in such areas and all necessary precautions must be taken.

Laboratory Procedures Including Taxonomy and Intercalibration

Analysis may be divided arbitrarily into four routines:

i) sorting (pretreatment, extraction, preservation);

ii) identification;

iii) recording;

iv) biomass estimation.

Sorting

In order to ensure the validity of results produced from a benthic survey, care must be taken at all stages of the procedure. Although sorting is probably the most mundane, laborious and onerous of the tasks, it is vital that it is undertaken efficiently and methodically.

Care must be taken in the use of biological preservatives, many of which have acknowledged health hazards and may interact adversely with other common laboratory chemicals. Staff handling such materials must be made fully aware of the dangers and suitable protective clothing and safety facilities must be made available (eg. fume hoods for formalin etc). The laboratory's codes of practice should be followed; reference to Collins (1985) and Baker and Wolff (1987) is also recommended.

A recommended procedure for sample processing is given in the following paragraphs. Methods for processing are also well documented in Eleftheriou and Holme (1984).

Pretreatment

The sample should be washed into a sieve, with a mesh at least as fine as that used in the field, and gently washed with copious amounts of water to remove the fixative/preservative under some form of forced ventilation. A number of different methods have been devised to simplify extraction. Experience has shown that for most practical purposes coarse elution can be recommended, as follows:

The sample is repeatedly agitated with fresh water and the supernatant liquid poured into a fine mesh sieve until no sample material is being washed off. This gives rise to a "light" fraction containing such groups as polychaetes and crustaceans, and a residual "heavy" fraction which retains molluscs, echinoderms and heavier–bodied members of other taxa. If desired, sieving through a series of meshes may be employed to allow easier sorting, and to avoid the shadowing effect of large particles over small individuals.

Alternative methods are available, which may be appropriate in some circumstances.

i) Mechanical elution may be recommended for large–volume shell gravel samples. It employs a fluidised sand bath (after Barnett; see Eleftheriou and Holme, 1984, p199) to elute the animals and, although the efficiency is claimed to be 98—100% for a 10 minute extraction, its use is rare.

ii) Flotation – various solutions that create density gradients are used to extract the fauna. Methods have been devised using colloidal suspensions such as Ludox–TM (Pfannkuche and Thiel, 1988). These techniques tend to be messy and cannot be recommended except for meiofauna.

The addition of the vital stain Rose Bengal can be recommended as a matter of routine, usually at the time of fixation in the field. None of the above methods can overcome the major problem of separating animals from fine particulate organic detritus. This may, to some extent, be overcome by the use of counterstaining techniques (Williams and Williams, 1974).

Extraction

The fauna and, if present, a representative sample of fruit seeds and other sewage–derived artefacts should be extracted against a suitable contrasting background with good illumination.

The extraction of the fauna from the sample or its eluted components can be undertaken without magnification if meshes of 1 mm or greater are used in the field sampling and residual material does not interfere with separation. Where finer meshes are used, some form of magnifying aid should always be employed, usually a binocular microscope, or an illuminated magnifier (x4). Following extraction, a second, less rigorous, sorting should be undertaken to ensure that no material has accidentally been missed.

Preservation

At this stage, the extracted animals should be separated at least into their respective groups, although more experienced workers may be able to separate at the family level and also separate out the most common species to ease later taxonomic analysis. If identification is to be undertaken immediately following extraction, then it may be permissable to sort directly into fresh water. However, if identification is likely to be delayed, then the various groups should be sorted into an appropriate preservative in a suitable watertight container. If prolonged storage is envisaged, these containers should be held in a vapour control cabinet or a percentage of glycerol should be added to the preservative to prevent drying out.

Suitable preservatives tend to be a matter of personal choice, but the relative characteristics of the preservative and preserved organism should be taken into account. Either buffered 5% formalin (2% formaldehyde) or a combination of 70% alcohol (90%) and glycerol (10%) can be recommended as appropriate.

All tubes (not lids), petri dishes and containers **must** be well labelled and a list of sorted samples and check–sorted samples, with initials, dates etc, should be maintained. This "sample history" is more important when a number of taxonomists, in a variety of locations, are employed to identify different groups.

Identification

Many standard taxonomic keys are available for the various taxa, but most are incomplete, either because outdated or owing to geographical limitations. However, mention should be made of the important contribution to UK marine taxonomy made in recent years by the Estuarine and Coastal Sciences Association (formerly the Estuarine and Brackish–Water Sciences Association (EBSA))/Linnaean Society series of keys. Inevitably much of the very recent taxonomic research material is contained within unpublished (grey) literature and, whilst it is difficult to maintain up-to-date information on all species and groups, some effort must be made to use current taxonomic texts.

In order to surmount some of these difficulties, the establishment of a formal system of communicating relevant up-to-date taxonomic literature to all interested parties is advocated. As a starting point to this, a bibliography updated from a 1988 Forth River Purification Board report (no TW5/88) is given in Appendix IV.

A recent workshop (Bayne, Clarke and Gray, 1988) showed that it was possible to discern the same division of stations using identification at the species, genus or family level employing a variety of multivariate techniques. These divisions were evident in situations where there were gross environmental differences between stations, and data are not at present available as to the sensitivity of the lower level of identification where only small, but possibly significant, differences might exist. Whilst the economic aspects of the less intensive identification approach might seem attractive to management, the case for undertaking full taxonomic analysis at least in baseline surveys is irrefutable. The possible use of less intensive taxonomy for ongoing surveys requires further evaluation.

An extremely useful method of standardising identifications can be found in the ECSA series of taxonomic workshops. Operators should be strongly encouraged to make full use of these taxonomic workshops in an attempt to rationalise identification between the various institutes involved in disposal site monitoring.

The use of a common nomenclature would be of great value in comparative work. The recently produced Species Directory to British Marine Fauna and Flora (Howson, 1987) might prove useful in this respect. It is the most complete listing currently available, and also contains taxonomic notes where there is some doubt about nomination. However, the coding system employed in this directory is not considered suitable for archiving purposes owing to its lack of a hierarchical structure.

Biomass Estimation

Estimations of wet weight (blotted) biomass, of both individual taxa and entire samples, should be routinely made for all quantitative monitoring surveys. These should be expressed as ash–free dry weights (AFDW) by drying to constant weight at 60°C and ashing at 520°C for six hours. Since the determination of AFDW will necessarily preclude future reference to specimens for taxonomic or other purposes, the use of conversion factors is recommended. Many of these can be derived from the literature, backed up where necessary by local calidation. Before biomass determination, samples should be left under preservative for a minimum of three months to allow for weight loss stabilisation (see, eg Brey, 1986; Duineveld and Witte, 1987).

Recording

There are many methods of initially recording identifications, ranging from note books to specially prepared sheets incorporating taxonomically arranged species lists. Such sheets have been criticised on the grounds that they could force identifications into the strictures of the chosen data sheet, and the more open (blank sheet) recording method is recommended. This is less likely to constrain the less experienced taxonomist.

The data recording should be as methodical and precise as possible, and should include the abundance and biomass of each taxon identified, along with any relevant taxonomic notes. Only animals with heads should be counted; colonial sessile epibenthic taxa (eg hydroids, bryozoans) should be recorded on a presence/absence basis. If no complete specimen of a taxon is taken, headless fragments should be recorded on a presence/absence basis but not included in the quantitative data analysis. The data should be recorded in such a manner as to facilitate quick and accurate entry into suitable computer formats.

A reference collection should be kept for each survey area and maintained in a normal curatorial manner. A selected classification text should be used to give a standard nomenclature (see p.14).

Intercalibration

Setting up an inter–laboratory calibration procedure may be costly in terms of time and resources, but this may be offset by the possible increase in confidence in the results if the venture proves successful.

Although intercalibration is not yet widespread, one laboratory (DAFS) is currently undertaking a major intercalibration exercise with a commercial monitoring contractor, in which all the technical aspects have been standardised, including survey strategy, gear used, sample number per station, taxonomic literature used, data analysis and report presentation. This exercise was not complete at the time of going to press, but at least the preliminary, theoretical problems have been overcome.

For those involved in sludge disposal site monitoring, it is proposed that a series of taxonomic intercalibration (ring test) exercises along the lines suggested by Ellis and Cross (1981) should be conducted in order to identify and then rectify any anomalies.

Objectives of Data Analysis

The objectives of the analysis of benthic data are:

i) To identify spatial patterns in the community under study and to relate these to other environmental characteristics, including the spatial extent of sludge dumping effects (exploratory and baseline survey data).

ii) To detect and quantify effects and temporal trends, if any, which are attributable to sludge dumping (ongoing survey data).

In order to answer these questions the data set provided by the benthic infauna should be ordered and analysed using a range of techniques, each of which will provide part of the necessary information. Most of the techniques referred to below are freely and widely available so that the mechanics of data analysis should be a minor part of the effort involved in any survey. This section proposes a basic suite of currently available statistical techniques for analysis of biological survey data obtained in sludge dumping ground monitoring, and makes general points about application of the methods, with the important rider that this is a rapidly developing field (eg Bayne *et al.*, 1988) and additions and improvements to analytical methods are commonplace. The value of parallel application of a range of other methods is also recognised wherever these help to elucidate structure in the data. For a detailed description of the methods and computer packages available, it will be necessary to consult the references quoted. Useful general texts include Sokal and Rohlf (1969), Green (1979), Gauch (1982) and Pielou (1977, 1984).

Data set

As well as biological data, analysis will usually involve consideration of physical/chemical data such as sediment particle size. Although the data may arise from a variety of survey techniques and may be in the form of counts or continuous variables (eg biomass), the standard approach given below will still apply. However, caution should be exercised in applying parametric statistics to count data; often, non–parametric methods may be more appropriate.

In general we are dealing with sample data from large populations and taxonomic data. Statistical methods used must take these factors into account. In particular, the importance of using a standard taxon list throughout any series of analyses in one area is stressed. Also, the use of statistical techniques on biological data must always be accompanied by expert biological interpretation of the results.

Developments in statistical analysis

Multivariate analysis is an active area of current research, and new methods and applications are appearing regularly. With this qualification, the proposals below are for methods which have gained, or are currently gaining, widespread acceptance and appear to be the best available at present for the analysis of dump site data (Gauch, 1982; Field, Clarke and Warwick, 1982; Roddie, 1986).

Many univariate and multivariate techniques will be applicable to both spatial and temporal trend analysis. For convenience, emphasis is placed on spatial comparisons in the following account. Some additional methods for temporal trend studies are given on page 19.

Computer packages

Supposedly identical analytical programs run on different computers have yielded different results in one intercalibration exercise (Shillabeer, in press). Effort must therefore be made to eliminate this potential source of error from computer packages before their widespread use.

Recommended Approaches to Data Analysis

Initial data processing

The data must be collated, preferably using a coding system based on hierarchical taxonomy, and in a form compatible with returns to regulatory authorities. A matrix should be constructed of taxon abundance per replicate sample, and should include quantitatively and qualitatively recorded taxa. Where required, a species/station matrix should be constructed after merging replicates. Similarly, a matrix consisting of the wet weight biomass of individual taxa (by sample and by station as required) should be constructed and then converted to AFDW biomass using appropriate conversion factors.

Where data transformation is helpful or necessary, the log (x + 1) or the root–root transformation can be used, provided the requirements of the statistical analysis are thereby satisfied.

Where truncation of the data to eliminate rare taxa is deemed appropriate, or where uncountable taxa such as colonial animals are excluded from the analysis, the criteria used should be explicitly stated.

The species recorded quantitatively should be ranked according to abundance, and the top 10 taxa, along with the taxa contributing to the top 50% of the community, should be separately listed for merged replicates. This allows an assessment of the major elements of the community at each station. The abundances and biomasses of different faunal components (eg opportunistic species, transition species, etc) should be examined.

Univariate methods and presentation of data

For grid survey data, contour plots should be prepared for primary variables (eg total taxa, quantitatively recorded taxa (S), abundance (A), biomass (B)) or derived variables (eg diversity indices, A/S ratio, B/A ratio).

A wide range of diversity indices have been employed in the analysis of ecological data. Limitations to interpretation which are inherent in the reduction of complex data to single figures should always be recognised. For routine purposes, the widely used Shannon–Wiener index (H') for sample data is considered to be the most suitable, in conjunction with calculation of evenness (J') (Pielou, 1975). However, the following cautions apply:

i) As H' is an information measure only, any taxonomic level or mixture of levels can be used, but it is essential to standardise the taxon list within a study and comparisons between studies must take this into account.

ii) H' may rise initially, then fall, with increasing impact of sludge dumping.

iii) H' itself has no biological significance and the same H' value may be obtained from totally different communities.

iv) H' is biased and assumes a random sample. It will usually rise with the number of samples, so a standard sampling procedure is essential.

v) Log_2 should be employed as the base in order to give a value for H' in "bits".

For transect data, gradient analysis should be used in which plots are made of primary or derived variables against distance from the point source (expressed as appropriate means with 95% confidence intervals). Since there is often good evidence for non–linear (successional) responses along such transects, this must be taken into account in planning the statistical analyses. For example, non–parametric testing of differences between stations based on lists of taxa ranked by abundance may often be appropriate.

In addition, other graphical expressions of the data may be used further to elucidate species abundance relationships between sites. Examples include rarefaction curves (Sanders, 1968; Hurlbert, 1971), k–dominance curves (Shaw, Lambshead and Platt, 1983), SAB curves (Pearson and Rosenberg, 1978) and ABC curves (Warwick, 1986; Warwick, Pearson and Ruswahyuni, 1987).

Finally, the use of analysis of variance (ANOVA) and analysis of covariance (ANCOVA), followed by multiple range testing to identify which site means are responsible for the ANOVA result, is advocated for evaluating the significance of patterns in the primary or derived variables. Suitable data transformation must be applied where necessary to achieve normality in the dependent variable.

Multivariate methods

As a means of reducing large taxon x station matrices to a less complex structure, the use of at least one ordination and one classification technique is necessary. However, it is stressed that these are heuristic, hypothesis–generating techniques, not hypothesis testing methods. It is essential that several multivariate techniques are used in order to assess the robustness of any associations derived.

Ordination seeks to represent relationships between samples (Q–mode) or taxa (R–mode) along a small number of axes in low–dimensional space on the basis of the taxonomic records alone. In Q–mode, the axes may then be related to environmental variables using well known statistical techniques, eg correlation/regression analysis, usually by non–parametric methods. This should be followed by testing of the hypotheses generated, using univariate or bivariate statistical methods such as analysis of variance. The use of R–mode analysis will assist in defining community compositions among sites.

Classification aims to divide samples (Q–mode) or taxa (R–mode) into groups such that within–group homogeneity and between–group heterogeneity are both large. The groups obtained can be compared with plots from ordination analysis and good agreement is usually obtained.

The following ordination techniques are recommended:

i) Detrended Correspondence Analysis (DECORANA) (Hill and Gauch, 1980), which is a development of Reciprocal Averaging (also called Correspondence Analysis). This is robust to non–linearity between the distributions of organisms and environmental gradients. Axes can be related to environmental variables.

ii) Non–metric Multidimensional Scaling (MDSCAL) (Kruskal and Wish, 1978). This makes very few assumptions about the linearity of relationships between taxon parameters and environmental variables and is therefore very robust but has a large requirement for computer time. MDSCAL is recommended as a complement to DECORANA.

The following classification techniques are recommended:

i) Two–way Indicator Species Analysis (TWINSPAN) (Hill, 1979), a polythetic divisive technique, has the advantages that information about each sample is retained throughout the analysis and that taxa and samples are classified in a single step, allowing identification of communities.

ii) Group Average Sorting of Bray–Curtis similarity coefficients (for quantitative data) or Jaccard coefficients (for presence/absence data), is a polythetic agglomerative technique which starts by clustering similar stations and progressively links these clusters (Clifford and Stephenson, 1975).

The "overlay" method (eg Field, Clarke and Warwick, 1982), in which environmental data are superimposed upon the output from station ordination or classifications, is advocated as a simple and easily interpreted approach.

Temporal trends

All of the above methods also have applications in temporal trend analysis. Some additional proposals are given below.

Analysis of variance should be used to assess year–on–year trends in primary or derived variables (subject to suitable data transformation as necessary), followed by multiple range testing. Where possible, other environmental variables should be entered into the analysis as covariates (Gauch, 1982; Jeffers, 1978). Use of two way ANOVA by year and station, and the study of interaction terms, should be used to provide indications of trends at impacted sites which differ from more general trends.

The application of time–series analysis methods can be recommended where cyclic trends have been identified in the biological variables. Appropriate methods have been reviewed by Gray, Bosch, Heip, Jones, Lassig, van der Horst and Wolf (1980).

Archiving

The future development of a standard computer package, providing a suite of archiving and analytical techniques appropriate to sludge disposal site monitoring, is considered to be highly desirable. Evaluation of taxonomic coding systems is required before further development and adoption. However, this system should be based on hierarchical taxonomy and be in a form compatible with annual returns to regulatory authorities

Reporting

All reports on sludge disposal ground monitoring should include relevant raw data and basic statistics as appendices, including details of subsampling procedures used. Simple graphical presentations should be used, where possible, to summarise major trends in the data. Each report should include an appendix containing taxonomic and faunal notes. All reports should stand alone without the need for reference to previous reports, and all reports should be freely available.

Conclusions

This review has dealt with a variety of approaches to field assessment of the marine benthic fauna, and has proposed standard methods for sampling and analysis. It also considers (in Appendix II) the potential utility of the benthic fauna for the derivation of Environmental Quality Standards for waste receiving areas, and proposals are made for the future evaluation of a number of methods which appear to show promise.

Studies of the benthic fauna in these areas represent only one of many possible aims in the determination of waste impacts on the marine biota. In recent years, a great deal of research effort has been expended in the investigation of parallel measures, employing either indigenous or transplanted species, and designed either as generic or pollutant–specific stress indicators. Recommendations concerning the utility of such measures for routine monitoring of sludge disposal grounds will be reported separately.

As yet these measures do not allow accurate predictions of wider impacts on indigenous communities, but their ultimate capacity to do so would clearly have implications for monitoring strategies, if only in terms of widening the scope of impact assessments. Future improvements in capabilities for monitoring the condition of biota in waste–receiving areas will require increasingly close cooperation between scientists engaged in field assessments notably of the benthic fauna and those involved with the development of parallel measures of other biological effects.

Because of the variable character of UK marine coastal areas it would be unrealistic to expect one type of benthic sampling equipment, or one standard survey design, to be applicable to all. Wherever possible, strict adherence to standard procedures is advocated, but it is also important that some measure of flexibility is retained within monitoring programmes, so as to allow for the future incorporation of demonstrable improvements in sampling efficiency or analytical methods. With this in mind, it is hoped that this account will provide the basis for improved coordination in the execution of benthic surveys at UK sewage sludge disposal sites.

Summary

1. This report outlines procedures for the conduct of surveys of the benthic fauna at sewage sludge disposal sites in the United Kingdom. The adoption of these procedures will be necessary for the eventual application of Environmental Quality Standards (EQSs) (see Appendix II).

2. Four stages are recognised in the development of a routine monitoring programme, namely: i) desk study; ii) exploratory survey; iii) baseline survey; and iv) ongoing survey.

3. The macroinfauna (ie animals living within sediments) are identified as the principal target for soft substratum areas and standard sampling methods are proposed.

4. In addition, semi–quantitative sampling of the epifauna (animals living at or just above the sea bed) using standard trawl sampling techniques is recommended.

5. The use of dredges and underwater photography may be particularly valuable for assessing the benthos of hard ground areas, but at the same time the need for further refinement and evaluation of the techniques is recognised.

6. Parallel measures of relevant physical and chemical properties should be routinely taken concurrently with the biological samples.

7. Standard procedures are given for laboratory processing, including sample extraction, identification and enumeration of taxa, preservation and biomass determination.

8. From a review of currently available techniques a suite of statistical methods for data analysis is proposed.

9. There is a need for development of a computer package for the statistical analysis and archiving of benthos data from UK sewage sludge disposal grounds.

10. Implementation of a research programme is advocated in order to evaluate the potential of benthic community data to be used to derive quantitative and qualitative EQSs.

11. Similarly, further research into the potential of alternative biological effects measures for setting EQSs is required.

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Appendix I

Recent Approaches to the Monitoring of Benthic Communities at UK Sewage Sludge Disposal Grounds

Introduction

This appendix reviews methods used currently and over the past 15 years in the biological monitoring of sludge disposal grounds and demonstrates the wide variety of techniques employed (Appendix Table I). The information has been compiled from published and unpublished reports and from personal comments. Although the coverage is not exhaustive, eg several of the early MAFF and DAFS surveys (such as those undertaken at the Plymouth, Bristol Channel and Lyme Bay sites) are not described in detail, the methods used were similar to those reported here.

It is apparent that the majority of surveys have concentrated on assessing variability across disposal areas and, in a few cases, a temporal analysis of the data has been carried out only retrospectively.

Frequency of Sampling

There have been very few regular (annual) monitoring surveys, but several of the disposal grounds have been monitored at infrequent intervals. Also the number of stations has often varied in successive surveys. For example, Inner Liverpool Bay (North West Water Authority (NWWA)) has been monitored approximately annually since 1972, the Tyne (Northumbrian Water Authority (NWA)) and Belfast Lough (Department of Agriculture, Northern Ireland (DANI)) have been, or are being, sampled approximately biennially, whereas the Bristol Channel (Welsh Water Authority (WWA)) site has been sampled much less frequently.

At the Forth (Lothian Regional Council (LRC)) and Bristol Channel localities, both foul and good weather sites exist — this has implications for the design of monitoring programmes. The two Forth grounds (Bell Rock and St Abb's Head) are monitored (by the Forth River Purification Board (FRPB) on behalf of LRC) at the end of a dumping cycle, ie at the time when any effects of dumping should be evident.

Bottom Substrata

Substratum type frequently reflects prevailing hydrographic conditions and it can be seen from Appendix Table I that most grounds have been chosen for their high-energy (dispersing) nature. One exception is Garroch Head, which is a low-energy (accumulating) site. However, several of the areas surveyed have muddy pockets which are potential "sinks" for contaminants.

At the St Abb's Head and Tyne grounds, muddy sands prevail. The Liverpool Bay ground and to a lesser extent the Thames site have coarse sand ribbons interspersed with a different sediment type. At other sites, eg Humber, Bristol Channel and Belfast Lough, substrata ranging from muddy sands to stones and even exposed rock can be found. As a consequence of this, sediment heterogeneity is a major source of variability in the faunal data.

Sediment Analysis

The sedimentary features of particle size and organic content are essential for the interpretation of the faunal data. With one exception (Garroch Head) all the programmes included particle size analysis (PSA) on a more or less regular basis, although the methods employed varied with survey.

The monitoring of the Garroch Head ground includes a sediment description of each station. In monitoring the Thames ground MAFF obtained a useful description of sediment colour using an arbitrary scale. The texture, colour, degree of bioturbation and smell are recorded at each station on the Forth grounds.

Organic matter was determined in most programmes, although the type of analysis differed with survey. The sediment redox potential characteristics were also assessed in many programmes on both dispersing and non-dispersing grounds. However, the coarse sediments at some grounds prohibits coring to provide undisturbed samples for redox potential analysis.

Proportion of Sampling Stations at which Benthos is Assessed

This has varied from 12 to 100% of the stations sampled. Recent programmes have assessed contaminants, both persistent ones and sewage debris over a wide area, while sampling the benthos at fewer sites (eg MAFF, Tyne, DAFS, and FRPB Forth grounds).

Sampling Gear Used

In general, the benthic monitoring programmes have used Day or van Veen grabs, although Smith-McIntyre and Hunter grabs have also been used. With very few exceptions, all programmes used a comparable size of grab of 0.1 m^2 area. Anchor dredges have been used in areas of coarse substrates (eg MAFF and WWA, Bristol Channel).

Many surveys have concentrated on spatial comparison; attempts at retrospective analysis of temporal changes using spatial survey data can be hampered by three notable potential sources of error:

i) differences in grab sampling design (eg a switch from Smith-McIntyre to Day grab); ii) differences in weighting, which can affect grab penetration depth, and therefore enumeration, and more significantly biomass estimates, of benthic organisms; and iii) differences in surface area sampled, eg MAFF used a 0.14 m^2 grab on some surveys, but have since reverted to 0.1 m^2.

Trawls and dredges have been used mainly to obtain biological material for contaminant analysis, although since 1986 Agassiz and otter trawls have been used to quantify the fish and epibenthic populations at the Forth grounds and their control sites. Similar work has recently been conducted at the Tyne site, using beam trawls. Video and still photography have been used on the Forth, Belfast Lough, Bristol Channel and Tyne grounds, in order to provide a visual assessment of the sites.

Faunal Component Sampled and Type of Data Produced

The macrofaunal populations were monitored on all grounds although the sieving mesh size used varied between 0.5 and 1 mm. Very few programmes have included the use of the true meiofauna (ie animals passing through 0.5 mm mesh sieves) as indicators of community stress, although the Scottish Marine Biological Association (SMBA) have incorporated this group as a research element, and both DAFS and MAFF have taken meiofauna samples for future reference if required.

Most programmes have obtained quantitative data. Where obtained, the production of "semi-quantitative" data has in some cases led to difficulties in interpretation, although this was unavoidable on, for example, hard substrata. Unusually, some studies produced quantitative data and then used binary data analysis techniques for their interpretation.

In earlier MAFF surveys of the Bristol Channel and Humber areas, only qualitative analyses of the benthic fauna were achieved, owing to sampling difficulties on the stony substrata.

Trawls and dredges may be used to produce quantitative or "semi-quantitative" data, though few programmes have attempted this (see above). In some surveys (MAFF, DAFS and FRPB) quantitative fish and epibenthic trawl data have been collected.

The Presence of Sewage Solids

This aspect has been reported separately (MPMMG, 1989) and so will not be discussed in detail here, although it should be noted that biological sampling provides an added opportunity for study.

Surprisingly few studies reported on the presence/absence of sewage-derived solid material in sediments (tomato pips, fibres, rags) on a routine basis. This may be regarded as a missed opportunity, since sediments are examined thoroughly during the field sieving and laboratory sorting stages. An initial analysis of Forth ground sediments indicated that the concentration of fruit seeds correlated with that of other faecal tracers such as coprostanol, although the latter analysis, performed by the Water Research Centre (WRc), was not carried out routinely.

The summer/winter sampling of the Forth grounds suggested that the number of the dominant sewage-derived solids and fruit seeds appeared to follow seasonal changes in human diet. Similar observations have been made the Tyne ground (MAFF). Furthermore, the estimation of these materials is unlikely to be reduced markedly with a change in mesh size from 0.5 to 1 mm. Finally, some programmes found the trawls or dredges useful for assessing the presence of larger artefacts (paper, rag etc).

Number of Stations and Months Sampled

The largest number of stations were sampled during research and/or baseline programmes (eg Liverpool Bay in 1970, Thames and Tyne grounds), and there was a tendency in many of the monitoring programmes to reduce with time the number of stations sampled. Several grounds (eg MAFF and University College of North Wales (UCNW), Liverpool Bay) were monitored with a sampling strategy that changed with time, thus leading to difficulties in extracting a time-series of data (see p.19). In order to overcome this problem, the UCNW programme used the same core-group of 18 stations located at or near the disposal area. The earlier Tyne monitoring data had to be adjusted to allow for laboratory/personnel changes (University of Newcastle/NWA), before temporal analysis was feasible.

As might have been expected from the largely spatial emphasis of earlier surveys, sampling has been carried out at all times of the year.

The Numbers of Samples per Station

The number of replicate samples taken per station has invariably been a practical compromise between the demands of statistical analysis and the resources available for sample processing. The asymptote on the cumulative species-sample curve often indicates the requirement for large numbers of replicate samples (eg >10) to represent fully the species complement in the benthic community. Regarding abundance estimates, even on grounds with homogeneous sediments, only very dense and uniform populations of any species will be accurately sampled by two or three replicate samples. Sediment heterogeneity at most grounds is cited as a major source of faunal variability between years.

In most programmes, either one or two samples were taken at each station. More have been taken in certain baseline or research surveys, although in some cases the extra samples were not analysed unless a later need was demonstrated. The Tyne (NWA) post-dumping strategy of taking five samples at each of relatively few stations facilitated the presentation and interpretation of temporal trends. In Liverpool Bay, only two samples per station were retained for benthos, but notes were made of extra samples taken for sediment analysis, so that comment could be made on the representative nature of the faunal grabs. In some programmes, the change over time in the number of samples taken per station could cause difficulties in temporal trend analysis.

Area of Disposal Site Relative to Total Survey Area

Some programmes (eg Bell Rock and St Abb's Head) have concentrated on near field effects, the area surveyed being a factor of only 2–4 times the size of the dump site. In contrast, the factor was 100—126 for certain "high-energy" grounds (Thames, Bristol Channel). Clearly, an assessment of local hydrographic conditions is required to determine realistic boundaries for baseline surveys. However, it may be noted that most documented effects on benthos have been found near to, or within, dump site boundaries, and that reduced spatial coverage may be permissible, following analysis of the results of an extensive baseline survey.

Mesh Size, Staining and Sorting of Benthic Samples

There was an approximately equal division between the use of 0.5 and 1mm mesh sieves, the choice depending on survey objectives, site characteristics and laboratory preferences. However, earlier in one programme (NWA, Tyne) the size of mesh differed along with the grab used. MAFF used a combination of 0.5, 1 and 2 mm mesh sieves at the Lyme Bay and Plymouth grounds to examine the effects of mesh size on interpretation of data. At the Tyne and Thames sites, MAFF have used a 1 mm mesh sieve for wide-scale descriptive surveys, and a 0.5 mm mesh sieve for follow-up sampling at fewer sites.

The majority of laboratories used vital stains to aid sorting and many sorted the samples under a binocular microscope (usually those sieved on 0.5 mm meshes). The practice adopted by DAFS of sorting fine mesh residues by eye and without staining is possible only with experienced staff. The fluidised sand bath was used by MAFF and UCNW for samples containing large amounts of gravel.

Data Analysis and Presentation

All programmes determined the species compositions and abundances, but biomass determinations were made routinely only for the Forth and Clyde grounds. The wet-weight (blotted) biomass of individual species was recorded by SMBA and DAFS, whereas FRPB recorded the biomass as wet weight converted to ash-free dry weight. The omission of such determinations in many programmes is perhaps surprising in view of the contribution they can make for relatively little extra effort to the interpretation of effects of organic rich wastes and to assessments of food chain interactions.

Methods for the interpretation and presentation of faunal data were varied. In order to detect spatial patterns within the data, multivariate statistical analyses (classification and ordination) were commonly used for grid sampling strategies. Data from transect sampling strategies (eg Garroch Head) were analysed by direct gradient analysis. The most commonly used method of classification analysis employed the Bray-Curtis similarity measure between station pairs, followed by group average sorting. Several ordination techniques have been used, of which multi-dimensional scaling (MDS), and detrended correspondence analysis (DECORANA: one of the Cornell University series of programs) are the most notable recent applications.

These and other recent developments in computer software can permit analysis of very large data sets produced from benthos monitoring surveys.

A wide range of diversity and evenness indices have also been employed, although the Shannon-Wiener information function was the most frequently used. A variety of other measures have been applied. Ranked dominance of species and ratios of abundance to numbers of species and biomass to abundance have been effective in analysing the Forth and Clyde data; however, such measures have not been widely used. The graphical description of community structure using rarefaction and k-dominance curves has also been used, eg for the Forth grounds.

Most programmes have concentrated on identifying spatial patterns but, in those cases where data were available, very few assessed temporal trends. One exception is the UCNW Liverpool Bay programme, where temporal trends in 180 species have been assessed after their abundances had been standardised to those expected in 100 grabs. Species were then categorised as common, rare, increasing or decreasing with time, and so on. Also, stations with similar characteristics were grouped together, and temporal trends in their community characteristics identified.

Relation of Benthos Data to Other Variables

Relatively few studies reported on a combined analysis of available physical, chemical and biological data, an omission which will limit the ability to explain benthos distributions. In one case (FRPB), the production of sample scores by ordination techniques allowed a rank correlation of faunal trends against environmental, physical and chemical parameters. A visual assessment of the relationship between different parameters using simple mapping techniques has been used in many surveys, eg MAFF.

Experimental Measures of Sub-lethal Stress

To date, such techniques have generally been employed in a research context, rather than routinely, by such organisations as MAFF, DAFS and WRc. The WRc studies have included:

i) Mussel embryo/larval bioassays on samples of a slick from a Thames sludge disposal vessel.

ii) Growth bioassay of the hydroid *Laomedia flexuosa*, the marine diatom *Phaeodactylum tricornutum* and embryos of the bivalve *Crassostrea gigas* using laboratory dilutions of, for example, Lothian sludge.

iii) "Scope-for-growth" of the mussel *Mytilus edulis* on the Plymouth, Bristol Channel and Thames grounds. MAFF and WRc have also undertaken an oyster embryo bioassay at a number of sites.

The effects of sewage sludge on fish and benthic invertebrates using mesocosms are being studies by DAFS (through DOE funding); experimental studies on the effects of sewage sludge on plankton are being carried out by Imperial College, London.

Appendix Table I

Biological monitoring practices on UK sewage sludge disposal grounds

Survey	Survey type/ frequency	Sediment type	Sediment analyses	Benthos stations	Sampling gear
1	Ma	Muddy sand	psa, Loi	9/21 *1	0.1 m^2 vv, Ag, tv occas
2	Ma	Coarse/med sand	psa, Loi	9/21 *1	0.1 m^2 vv, Ag, tv occas
3	Ma *2	Muddy sand	psa, C, Chl+pha, red	9/17	0.1 m^2 vv or S-M; AG + tv
4	Ma *2	Coarse/med sand	psa, C, Chl+pha, red	9/17	0.1 m^2 vv or S-M; AG + tv
5	Ma *3	Sandy/silt	psa, protein, Loi	7/38 *4	0.1 m^2 vv or Day
6	Ma *5	Sandy/silt	psa, protein, Loi	All	0.1 m^2 vv or Day
7	Mo	Predom fine sand	psa (sand), C/N	10/85	0.1 m^2 Day, beam trawl
8	Mo	Predom fine sand	psa	61/~100	0.1 m^2 Day
9	R	Fine sand, variable	psa, C/N	All	0.1 m^2 Day
10	Mo/R	Med - fine sand, mud pockets	psa, C/N, red	166/235	0.1 m^2 Day
11	Mo	Mud, sand-gravel + rock	psa	All	7.5 litre sample by Anchor dredge
12	Mo	Mud, sand-gravel + rock	psa	All	7.5 litre sample by Anchor dredge
13	Mo *6	Sand or mud/shell/sand/mix	psa, C/N, red	All	0.1 m^2 or 0.14 m^2 Day
14	M/Ra *7	Sand or mud/shell/sand/mix	psa, Loi	All	0.1 m^2 S-M or Day *8
15	Ma/R	Muddy seds	C/N, red, sed des	15/61 *9	0.1 m^2 vv, Ag, Ott, tv *10
16	M/R *11	Med-shell/gravel-rock	Psa, red at surface	All	0.1 m^2 Hun, beam, Ott, dredge, tv *10
17	Ma	Muddy sand + rock	C, N(K), psa	11/26	0.1 m^2 vv, Ott, Ag
18	Ma	Coarse/med sand	C, N(K), psa	9/26	0.1 m^2 vv, Ott, Ag

Please refer to the table of abbreviations (p.35) and the (*) footnote list (pp.36 and 37) to aid interpretation of this table

Appendix Table I (continued)

Survey	Biological data	Fauna	Sewage solids	No. biological stations month sampled	Replicates/ station	Dump area/survey area km²	Mesh/stain/sorting
1	Q	M	Na	9, May *1	2	20/36	0.5, rb, es
2	Q	M	Na	9, May *1	2	20/36	0.5, rb, es
3	Q *12	M *13	*14	9, *15	2	9/36	0.5, none, es *16
4	Q *12	M *13	*14	9, *15	2	9/36	0.5, none, es *16
5	Q	M	Na	53→ 5 *3, 17	2→10 *17	6.6/170	1, rb, es
6	Q	M	Na	7 *5	5	6.6/170	0.5 or 1 *18, rb, es
7	Q *19	M	*20, 21, 22	10, Feb	3	6.6/*23	0.5, rb, bs
8	Q	M	Na	61, Feb	1	6.6/130	1, e, es
9	Q	M	Na	74, July	4 *24	7.6/783	0.5, none, bs
10	Q	M	*25	166, Apr/May	1	7.6/345	1, e, es
11	S-Q *26	M	Na	51, June	1	8.7/1100	1, none, es
12	S-Q *26	M	Na	15, July	1	8.7/1100	1, none, es
13	Q	M	Na	41-63, Sep-Nov *27, 28	1-2 *29	59/786	1, e, es
14	Q	M	Na	16-22, Autumn *27	5→2 *38, *17	59/527	1, none, sb + es
15	Q *19	M *30	Na	15, May *31	1 *32	2.2/48 *33	1, rb, es
16	Q	M	*21	25-30 *11	1	6/50 *34	0.5, rb, bs
17	Q *12	M	*22	11, June	2	20/36 (*35)	0.5, rb, bs *36
18	Q *12	M	*22	9, Oct-Jan	2	20/36 (*35)	0.5, rb, bs *36

Please refer to the table of abbreviations (p.35) and the (*) footnote list (pp.36 and 37) to aid interpretation of this table

Appendix Table I (continued)

Survey	S/A/B	Faunal data analysis	Bio/sed correl	Comments	Survey
1	S/A	Com des, Div, RD, C, O	No	Agassiz for bioaccum work	IOE, St Abbs '82-85
2	S/A	Com des, Div, RD, C, O	No	Agassiz for bioaccum work	IOE, Bell Rock '82-85
3	S/A/B	Com des, Div, RD, C	No	Fish for bact anal	DAFS, St Abbs
4	S/A/B	Com des, Div, RD, C	No	Fish for bact anal	DAFS, Bell Rock
5	S/A	*37	No)	Fish bioaccum + microbiol	NWA, Tyne '73-80
6	S/A	*37	No)	done by MAFF	NWA, Tyne '81-83
7	S/A	Com des, gradient anal	Yes	Bact of seds	MAFF, Tyne, '84
8	S/A	Com des, C	Yes	Bact of seds	MAFF, Tyne '82
9	S/A	Com des, C, O	Yes	Bact + faecal indicators of seds	WRc, Thames '85
10	S/A	Div, C, O	Yes	Bact of seds	MAFF, Thames '77
11	S/A *26	Com des	No)	High energy area; also fish	Wessex Water, Bristol '81
12	S/A *26	Com des	No)	dynamics assessed	Wessex Water, Bristol '82
13	S/A	Div, C, O	Yes	Bact of seds	MAFF L'pool Bay '75-80
14	S/A	Com des, C, O	No	Drifter work to define risk area *39	UCNW L'pool Bat '70-88
15	S/A/B	Com des, grad, R	No	Bact and histopath on fish	SMBA, Garroch Head '79-88
16	S/A	Div, Twin	Yes	Bact of fish and shellfish	DANI, Bel Lough '85-86
17	S/A/B	Com des, Div, RD, C, O, R	Yes)	Bact and histopath on fish + fish	FRPB, St Abbs '86-88
18	S/A/B	Com des, Div, RD, C, O, R	Yes)	and epibenthos populations assessed	FRPB, Bell Rock '86-88

Please refer to the table of abbreviations (p.35) and the (*) footnote list (pp.36 and 37) to aid interpretation of this table

Appendix Table I (continued)

Abbreviations

Column	Abb	Explanation
Survey type/ frequency	M	Monitoring
	R	Research
	a	Annual
	o	Occasional
Sediment analyses	psa	Particle size analysis
	psa (sand)	Psa excluding silt/clay
	Loi	Loss-on-ignition organic matter
	C	Carbon by wet oxidation
	N(K)	Nitrogen (Kjeldahl)
	chl	Chlorophyll a analysis
	pha	Phaeophytin pigment analysis
	C/N	Carbon, nitrogen by analyser
	sed des	Sediment description (subjective)
	red	Redox potential
	Ben stns	Stations at which benthos sampled
Sampling gear	vv	Van Veen grab
	S-M	Smith-McIntyre grab
	Day	Day grab
	Hun	Hunter grab
	Ag	Agassiz trawl
	Ott	Otter trawl
	Beam	Beam trawl
	Dredge	Scallop dredge
	tv	Underwater television
Biological data	Q	Quantitative data
	S-Q	Semi-quantitative data
Fauna	M	Macrofauna
Sewage solids	Na	Tomato pips, sewage solids etc not assessed
Replicates/station		Replicates per station
Mesh/stain/ sorting	rb	Rose-bengal stain used
	e	Eosin stain used
	es	Sorting by eye
	bs	Sorting by binocular microscope
	sb	Sorting by fluidised sand bath
S/A/B		Species/Abundance/Biomass recorded
Faunal data Analysis	Com des	Community description given
	Div	Diversity indices used
	RD	Species ranked dominance given
	C	Classification analysis used
	O	Ordination analysis used
	g	Gradient analysis used
	R	Size-ratio and abundance ratio analysis used
	Twin	Twinspan used
Biol/sed correlation		Statistical analysis of biological, physical and chemical data as a single data set
Comments	Bact	Examination for faecal bacteria
	Bioaccum	Accumulation of contaminants in biota
	Histopath	Pathological signs assessed

Appendix Table I (continued)

Footnotes (*)

1. Number of stations being increased to 13.
2. 1978, 1979, 1980 as post-dumping; area also surveyed pre-dumping.
3. Pre-dumping surveys — spring and autumn sampling.
4. A baseline benthic survey of 53 stations was originally sampled.
5. Post-dumping surveys — spring and autumn sampling.
6. 1975, 1976, 1978 and 1980 samples taken on and around the dump ground.
7. 1970 base survey; more or less annual monitoring since 1972.
8. TV work; Agassiz trawl used occasionally but not reported in detail.
9. 1979 —1983, 24 stations monitored; 15 stations monitored in 1984; 1985-onwards, variable number of stations monitored depending on trends found.
10. TV study occasionally.
11. Three surveys over 12 months (March, August/September, March).
12. Semi-quantitative Agassiz trawl work at each station.
13. Meiofaunal samples taken not worked up.
14. Tomato pips assessed in 1980.
15. Benthos sampled June 1978; April and October 1980; April 1979 but Agassiz trawling was also done in April and October 1979.
16. 0.06 mm mesh and elutriation for meiofauna.
17. The arrows denote a change with time.
18. The grab type depended on vessel used. Mesh size was 1 mm for broad grid surveys, 0.5 mm for ongoing surveys at seven stations.
19. Semi-quantitative data for beam trawl work.
20. Sludge debris was taken in beam trawl samples.
21. An assessment of tomato pips in sediment and sewage debris in the dredge samples.
22. Sewage debris and fruit seeds noted in sediment.
23. A transect parallel to the coast was used for benthos work; a grid of stations was retained for sediment work.
24. Not yet known if all replicates will be analysed.

Appendix Table I (continued)

25. A subjective assessment of sediment colour (an arbitrary scale) was used and then incorporated into the faunal data analysis.

26. A semi-quantitative abundance scale was used for the benthos.

27. The number of stations sampled varied with each year.

28. A survey of 28 stations to the east of the spoil ground took place in 1977.

29. Two replicates were taken with the 0.1 m^2 grab but only one with the 0.14 m^2 grab.

30. Meiofauna assessed as research project.

31. Five Agassiz and two other trawls also taken.

32. An extra grab was taken as back-up.

33. A cruciform-transect pattern is used for the benthos rather than a grid-pattern; latter used for sediment samples.

34. The stations are concentrated towards the spoil site.

35. Control station included 5.5 km from centre of dump site.

36. Binocular sorting of 0.5 —1 mm fractions; >1 mm by eye + simple elutriation technique.

37. A rationalisation of the data had to be performed owing to the number of labs/personnel changes involved in the data collection. A time-series data set was extracted and analysed by multi-variate techniques.

38. Five replicates in base survey, one in monitoring, average of 50 grabs pa.

39. A close group of stations, at the northern part of the spoil-ground, was used as replicates for time-series analysis.

Appendix II

Environmental Quality Standards for the Benthos at Sewage Sludge Disposal Sites

Introduction

Environmental Quality Standards (EQSs) are a familiar tool in UK waste disposal management, entailing the setting of limit values for a range of contaminants in receiving waters as an aid to the control of discharges. Depending on the nature of the discharge(s), these limits are usually determined according to local objectives for the maintenance or improvement of water quality, and are notably dependant on the uses made of the recipient areas, eg for fisheries, recreation or abstraction for water supply.

The determination of EQSs for biological variables in marine areas is largely untried for a variety of reasons, and the purpose of this appendix is to address the potential for future application. The account begins with a review of the limited literature on this topic, and then presents a rationale for a research programme geared to EQS derivation and testing. Detail is then provided on five methods of quantifying attributes of benthic community structure, potentially useful for this purpose. The implementation of standard procedures for sampling and analysis is a pre-requisite for an effective outcome. An assessment of the potential role of alternative biological effects measures in standard setting at this stage in their development is also included.

Finally, the use of a hypothetical standard based on a comparison of treatment and reference sites is considered, in order to highlight, firstly, potential advantages and limitations attached to the application of an EQS approach using benthic data and secondly, the importance of validatory research before implementation.

Review of Literature

A literature search of eight databases (Aquaculture, Aqualine, Aquatic Sciences and Fisheries Abstracts, Biosys, Fish Laboratory (Lowestoft), Oceanic Abstracts, Water Resources Abstracts, Waternet) identified a number of papers, notably from the USA, which have critically reviewed the philosophical background to field monitoring of biological effects, and dealt with the issues of survey design, sampling methods and what may or may not be feasible in future applications. However, hardly any publications on routine applications of EQSs for benthos exist. Thus there are no well established precedents to draw from regarding the marine benthos.

Despite this, there have been numerous attempts, based on empirical findings, to categorise the response of marine benthos to varying degrees of pollution. Regarding organic enrichment, the most notable exercise is that of Pearson and Rosenberg (1978), whose model for successional changes in communities — summarised in SAB (Species, Abundance, Biomass) curves — has been found to be widely applicable.

Several authors have proposed single figure indices in an attempt to fulfil requirements for an easily understood summary of biological effects upon which waste management action can be taken. The ability of such indices adequately to quantify ecosystem responses remains an area of much contention, as is aptly illustrated by the quotations from two recent scientific papers.

Green (1984) wrote "...no ecologist should encourage a non-biologist manager or politician who wants to search for that elusive goal, the single variable that can be easily and inexpensively determined and that represents human impact on natural ecosystems". Boesch (1984) wrote "The ecologist should not be blamed for the fact that environments deteriorate in complex and subtle ways as a result of human activities".

In fresh water systems, a number of simple schemes for linking benthic changes to water quality have been evolved in recent years. This is largely a consequence of the enclosed nature and unidirectional flow of rivers, allowing the effects of pollution and their spatial extent to be more easily identified.

Perhaps the most notable recent effort towards the development of quality standards for the marine benthos has been associated with the study of deep water sewage discharges off the Californian coast (the "Southern California Coastal Water Research Project"). Word (1979) proposed an "Infaunal Index" which was based on the concept of indicator species, identified from empirical observations on their occurrences near to and distant from outfalls. Three divisions in the benthic habitat were recognised, namely "normal", "changed" and "degraded". A considerable amount of effort was involved in defining a "normal" fauna - entailing sampling at several hundred coastal and offshore sites in the general vicinity. Thus the "norm" is site-specific, as are the lists of indicator species, though a number of taxa which they represent are cosmopolitan.

The definition of an uncontaminated "norm" for a number of inshore habitats around the UK in receipt of sewage sludge could be problematical and this is reflected in the EQS proposals (below). For example, there is no obvious equivalent to the outer Thames estuary for comparative purposes. However, it is worth noting the use by Kingston (1987) of an empirical standard derived from pre-operational benthic surveys around oil platforms for unpolluted offshore northern North Sea waters using the Shannon-Wiener diversity index.

Other examples of attempts to quantify benthic responses in the field to disturbance/pollution (in such a way as might loosely conform with the requirement of an EQS) include the successional model of Rhoads and Germano (1986) which is analogous to that of Pearson and Rosenberg (1978). Leppakoski (1975) defined a "Benthic Pollution Index" based on the spatial extent of zones affected to different degrees in Swedish and Finnish waters. A development of this approach, involving the biological classification of intertidal areas in order to calculate a "Benthic Quality Index", was proposed by Jeffrey, Wilson, Harris and Tomlinson (1985).

The fact that none of the proposed indices of environmental degradation has yet found universal application appears to be due to the over-riding constraints on their performance imposed by the influence of local factors (notably the nature of the habitat and contaminant loads). This is in agreement with the observations of Pearson, Gray and Johannessen (1983). It is therefore concluded that the production of an absolute quantitative standard describing the health of the benthos of inshore UK waters is not at present feasible. However this does not preclude alternative approaches to evolution of quality standards, as will be discussed below.

Rationale for the Future Establishment of Environmental Quality Standards Using Benthos at Sewage Sludge Disposal Sites

Two lines of investigation are most promising. The first is the comparative evaluation of "treatment" and "reference" sites, in which the latter are distant from the influence of the point source of interest. Note that often the reference sites will not be representative of uncontaminated environments. Hence the term "reference" is considered to be more appropriate than "control". The work of Skalski and McKenzie (1982), in which they advocate pairing of all "treatment" sites with environmentally similar "control" sites ("control/treatment pairing"), provides a useful illustration of such an approach applied to benthic counts (see also p.46). The second is the evaluation of gradients of response along station sequences at environmentally similar sites extending beyond the anticipated zone of impact. The statistical analysis of such gradients which will be required in order to derive quantitative standards is less well developed.

Note that in both these lines the objective is to detect statistically significant change (eg in benthic counts) in response to sludge inputs. Such an objective is arbitrary, in the sense that the quality implications of such change is not directly addressed. The formulation of parallel qualitative standards, designed to aid the expert interpretation of the significance of such changes to the local environment, will therefore be desirable.

The understanding of marine ecosystems is not sufficiently advanced to allow unequivocal identification, and then quantification, of critical states along the path of any degradative process within subsystems (eg benthos) beyond which wider changes of an unnacceptable nature may be triggered. The main reason for this is self-evident: in contrast to many enclosed fresh water systems, the marine environment is essentially open in nature. Thus, for example, most commercial fish species are not narrowly territorial and have the capacity to avoid unsuitable areas. Also, the study of dispersion and effects of waste discharges, both in the water column and at the sea bed, is greatly complicated by tidal and residual currents.

A research programme is required to derive and evaluate appropriate measures, prior to routine application. This is for two main reasons. Firstly, the absence of a well-established precedent for such an approach has earlier been noted. There are too many uncertainties to allow immediate and uncritical application. Secondly, a good deal of research effort is currently under way to evaluate the suitability of different environmental measures of biological effect and the most suitable methods for data analysis (exemplified by a recent Workshop; see Bayne, Clarke and Gray, 1988). The results are only now becoming available and need to be critically assessed.

The proposed research programme will therefore involve i) the establishment of reliable methods which will signal the onset of change and ii) the linking of such change to ecosystem quality, eg contaminant inputs, valued or other resources.

Proposals for Future Investigation

Multivariate techniques

The large amount of data on benthic communities generated by ecological studies requires the use of multivariate statistical techniques such as ordination and classification to determine patterns in the data. The use of these techniques is now widespread in benthic ecology but until recently they had not been used in a predictive sense.

A major research effort by the Freshwater Biological Association has recently produced a high quality database which has been used to link the faunal community with the environmental variables at any particular site (Moss, Furse, Wright and Armitage, 1987). Given the wealth of data for shelf benthos a similar approach could be taken and developed to provide initially qualitative and ultimately quantitative EQSs.

Method

In the FBA scheme, sites were ordered using detrended correspondence analysis (DECORANA) and then classified by two-way indicator species analysis (TWINSPAN), ie the sites are grouped according to faunal characteristics and the "indicator" species of the communities were determined. The site groupings were linked with environmental variables using multiple discriminant analysis (canonical variate analysis). In any future use of the scheme the data can be treated at the presence/absence or quantitative level and at any taxonomic level, although only high resolution produces the best predictive capability. In general, common trends in the faunal data will be derived and any deviation is determined from the "expected" community under given environmental conditions.

Proposed quality standard

As these multivariate techniques result in a series of site groups, a standard could be based on the percentage change in site group membership or as the movement of groupings along axes produced by the techniques. This type of analysis does not rely on the inclusion of physico-chemical data.

By employing the technique used by the FBA scheme, it may be possible to predict the general structure of a community using the physico-chemical data. Also, multiple regression analysis has potential for predicting the size of communities to be expected under certain environmental conditions. A standard could then be derived by comparing actual and predicted communities.

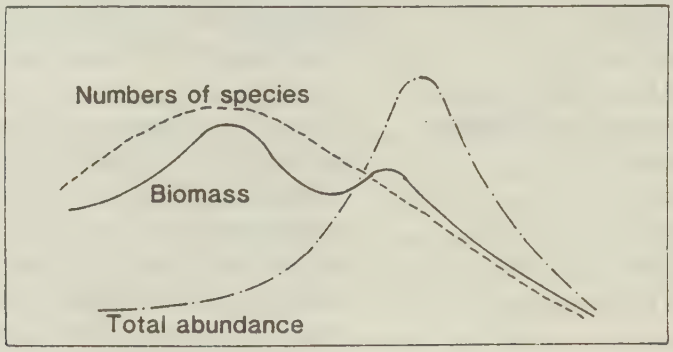
A TWINSPAN analysis followed by a Multiple Discriminant Analysis could be used to identify important determinands. The distribution of these determinands within site groups can then be examined and will lead to the derivation of ranges and thresholds. In this way, physico-chemical EQSs can be derived using biological data as a basis.

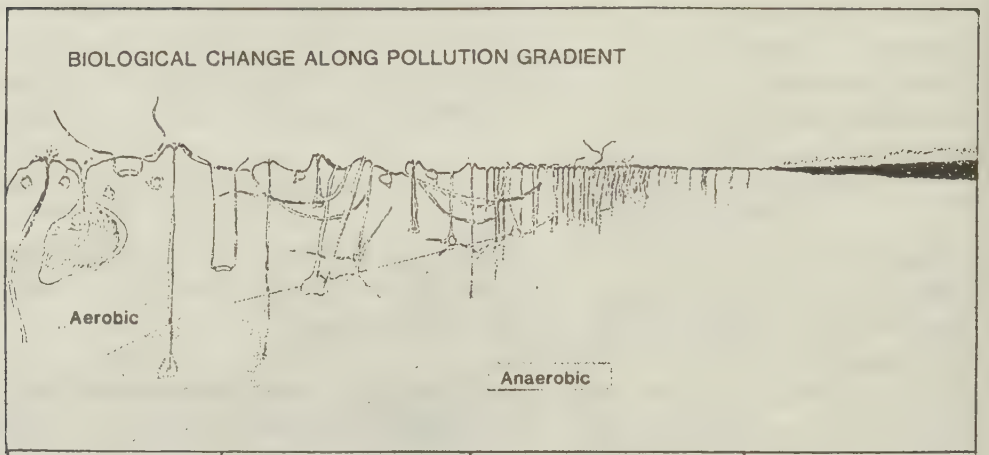
This approach has potential for setting EQS as community change attributable to environmental changes. However, any deviation may be difficult to quantify and the scheme would be more complicated in the marine field than in uni-directional fresh water systems. A desk study would show the potential for this approach and assess the need for further data to be collected.

Species/abundance/ biomass (SAB) curves

The faunal analysis of any benthic sample yields three basis statistics, S, the number of species present in the sample, A, the total number of individuals in the sample, and B, the total biomass of the sample. The values of these statistics are known to vary in a characteristic manner along any gradient of organic enrichment (Pearson and Rosenberg, 1978) as illustrated in Appendix Figure 1, thus reflecting the continuous change in community structure along such gradients. A comparative evaluation of these statistics from sample to sample may therefore be used to assess the relative degree of enrichment of any particular sample or area. The values of S, A and B may be plotted and compared separately or used to compute two derived ratios *viz* A/S, the abundance ratio, being the mean number of individuals per species in the sample, and B/A, the size ratio, being the mean weight of each individual in the sample (Pearson, Duncan and Nuttall, 1982).

These ratios also vary characteristically along enrichment gradients (Pearson, 1987) and, because of their intrinsic biological significance, are particularly useful in interpreting the effects of pollutant impact.





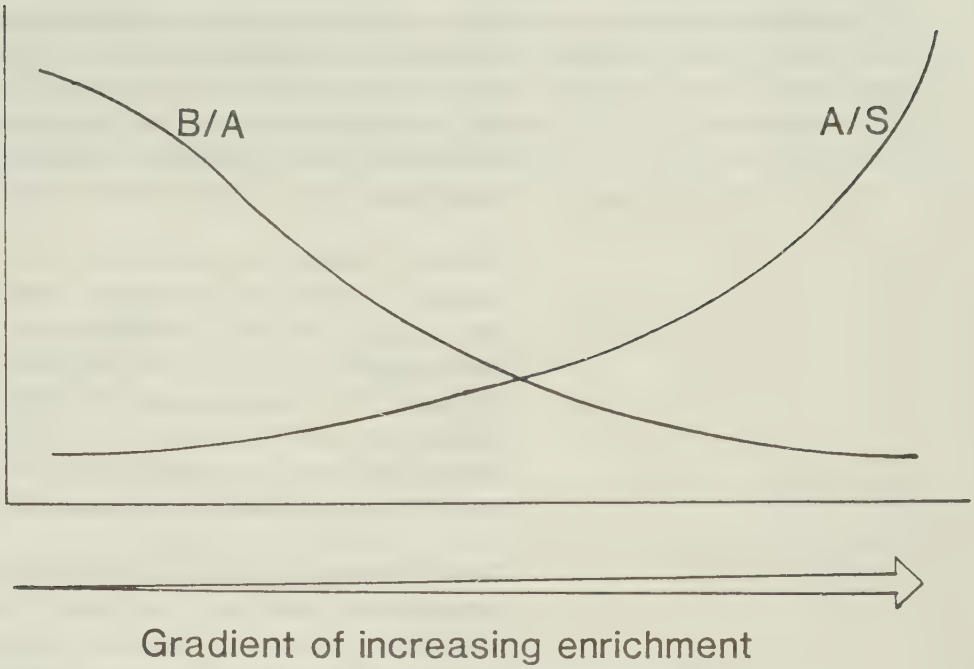
Normal	Transition	Polluted	Grossly polluted
	Increased abundance	Pollution indicator species	No Macrofauna

Appendix Figure 1. **Generalised diagram of changes in fauna, sediment structure and benthic community parameters along a gradient of organic enrichment (after Pearson and Rosenberg, 1978).**

Method

The characteristic SAB curves illustrated in Appendix Figure 1 are derived by plotting the values of each statistic as the ordinate against the degree of enrichment (measured as distance from a point source input or any convenient organic sedimentary parameter) as abscissa. For convenience of comparison the plots may be overlaid by selection of appropriate scales for each statistic as in Appendix Figure 1. In such plots S is lowest at the highly enriched end of the gradient, rises rapidly as enrichment declines, reaches a maximum in moderately enriched areas, and then declines to an intermediate level in unenriched normal communities. A is highest in highly enriched areas and declines progressively as enrichment declines. The B curve exhibits two peaks, associated with the high abundances in the highly enriched areas and the high diversities in the moderately enriched areas respectively.

Appendix Figure 2 illustrates a similar idealised plotting of the size and abundance ratios. These twin curves are somewhat more easily interpreted than the SAB plots as they demonstrate inverse properties along the enrichment gradient. A/S is maximal in the highly enriched areas and minimal in unenriched areas where B/A takes the reverse form.



Appendix Figure 2. Model curves for the abundance ratio (A/S) and the size ratio (B/A) along an idealised gradient of organic enrichment.

Proposed quality standard

Semi-quantitative standard

The changing shape of the curves along enrichment gradients can be used to assess the degree of enrichment from a point source by comparison with the idealised model curves in Appendix Figures 1 and 2. Moreover, any particular sample may be placed in an appropriate position along the enrichment gradient according to its SAB values. Subsequent deviation from that position will suggest either an improvement or a deterioration in the enrichment situation at that particular point. The exact shape of the curves, including the maxima and minima of each statistic, tend to be site specific and thus adequate interpretation is dependent on good baseline information.

Quantitative standard

Two approaches have been taken to developing quantitative standards from SAB curve models. Swartz, Cole, Schults and DeBen (1986) assessed changes in benthic and sedimentary conditions off sewage outfalls in southern California by using numerical classification techniques to identify clusters of samples in both time and space. These clusters were then identified with areas of "degradation" and "stimulation" by reference to the mean SAB values of the samples within each cluster and their relative position on the model SAB curve. The SAB values of each cluster were then compared statistically to establish the numerical significance of the observed differences relative to background conditions.

Smith and Bernstein (1985) developed a single figure index, based on multivariate (ordination) techniques, for quantifying benthic community change along enrichment gradients. The index was calibrated by reference to idealised and actual SAB curves and values. The authors claim that the index is sensitive to small community changes and capable of distinguishing between pollution effects and changes caused by natural gradients.

Both these approaches have considerable potential for setting quantifiable quality standards, but require further development and critical evaluation in relation to other possible methodologies.

k-Dominance curves

Graphical methods of illustrating community structure have been long used to compare the diversity of different samples in which the size of the sample or the number of individuals varies. The techniques are based on the fact that as sample size increases individuals are added at a decreasing logarithmic rate. In essence, the relative diversity of samples is assessed by comparing their curves describing the individuals/species relationship.

Sanders (1968) and Hurlbert (1971) described rarefaction curves, the number of species encountered when sampling increasing numbers of individuals. Recently, k-dominance curves have been proposed as a computationally simpler and more easily interpretable method of describing the structure of a community in relation to environmental stress (Shaw, Lambshead and Platt, 1983; Lambshead, Platt and Shaw, 1983). The k-dominance curves are a precursor of the ABC method (Warwick, 1986; see below) but do not rely on biomass estimations.

The k-dominance curves are characteristically of either a decreasing exponential curvilinear form when untransformed axes are used or a sigmoid form when a log-transformed abscissa is used. The shape and position of the curves are used to assess the structure of the community.

Proposed quality standard

Semi-quantitative standard

The temporal or spatial change in the shape of the curve will indicate the changes in the community. An increase in the abundance of the dominant (possibly opportunistic) species, a change in the abundance of transition species and a reduction in rare species will raise the curve. Comparison of the disposal site community with that of any reference site can then be made using the k-dominance curves. The changes in the central part of the curve can be also used as part of the approach of selecting particular species for study (Gray and Pearson, 1982).

Quantitative standard

In order to quantify the change in curve shape as a response to environmental change, it is proposed that changes are determined in the parameters of the mathematical representations (regression equations) of the curves. The simplest approach is to transform the curves to straight lines. Then, it is hypothesised, in the early stages of organic enrichment, as the diversity first increases slightly and then starts to decrease, the slope of those lines will remain the same but the intercept on the ordinate will firstly decrease and then increase. Eventually, with continued organic enrichment, the loss of species will cause the slope of the line to increase.

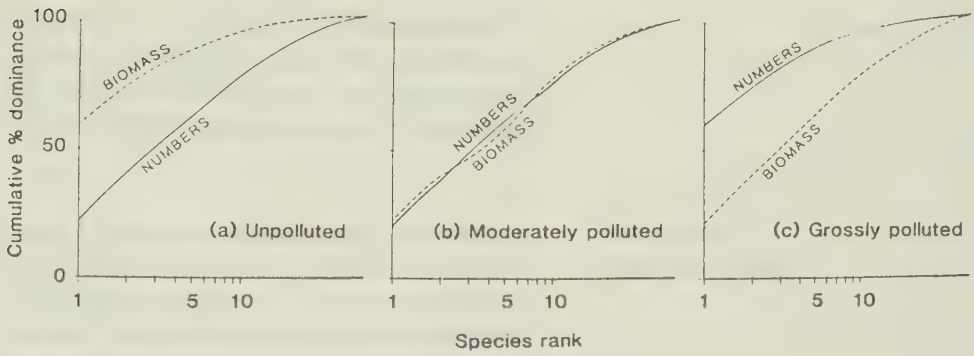
The k-dominance method may have potential for providing a quantitative benthic quality standard. The limits of its variability are not yet fully defined but data are available to detect its sensitivity and calibrate the method. Further work is needed to produce linear transformations of the curves but this is not difficult mathematically.

Abundance/biomass comparison (ABC) curves

This is a graphical method which exploits the differences in the distribution of individuals amongst species and of biomass amongst species in communities undergoing pollution disturbance.

Superimposed k-dominance plots (described above) for species biomass and abundance change in configuration along organic pollution gradients. In samples from enriched areas the biomass curve lies clearly above that of abundance owing to the dominance of relatively large organisms.

As organic enrichment increases the mean size of the constituent organisms in the community decreases; the biomass and abundance k-dominance plots approach each other and overlap at moderate levels of enrichment. At high enrichment levels, when the communities are dominated by large numbers of small organisms, the plots separate out once again with the abundance plot lying clearly above that of biomass (Appendix Fig. 3).



Appendix Figure 3. Hypothetical K-dominance curves for species biomass and numbers, showing unpolluted, moderately polluted and grossly polluted conditions.

Proposed quality standard

Semi-quantitative standard

The relative position of the abundance and biomass curves denotes the degree of pollution/enrichment of the community, and can thus be used as a visually assessed quality standard (Warwick, 1986).

Quantitative standard

As with the standard k-dominance curves it should be possible to transform the biomass and abundance curves into straight lines and, by a comparison of their slopes, quantify their numerical relationship. Such developments have not yet been attempted.

Evaluation

This extension of the k-dominance method has the advantage of relative simplicity and, with some development of an appropriate means of quantification, may have potential for setting quality standards. Following appropriate development, it would necessarily have to be evaluated in comparison with the other suggested methods.

Diversity indices

Measures of community structure such as diversity (H') and evenness (J') may be used to define norms against which changes due to waste disposal can be assessed. The chief limitation to such measures is the loss of information resulting from the condensing of complex data to single figures, which may even lead to misleading interpretations being made.

Proposed quality standard

In practice, it would be difficult to arrive at an absolute measure to quantify the pre-disposal norm at most inshore sludge disposal sites (see page 39 above). However, such indices may be useful as relative measures of change, when compared with one or more reference sites, and may find application along the lines suggested below.

Field bioassays: state of the art

Alternative Biological Effects techniques as opposed to the more classical macrobenthic assemblage techniques could be used in the context of setting EQS values for sludge disposal grounds. Their details will be reported elsewhere.

The techniques range from laboratory toxicity tests, which may be carried out on sediments collected from the sludge disposal ground (SDG) or on water samples taken during or after sludge disposal, to physiological responses of organisms transplanted to monitoring sites in and around the disposal ground. Many of the techniques have had limited application and at the present time insufficient data are available on which to base standards.

The database available from the results of benthic surveys is considerable in terms of the range of disposal grounds investigated and the time scale over which the data have been collected. This clearly gives a foundation from which to propose preliminary EQSs.

Proposed quality standard

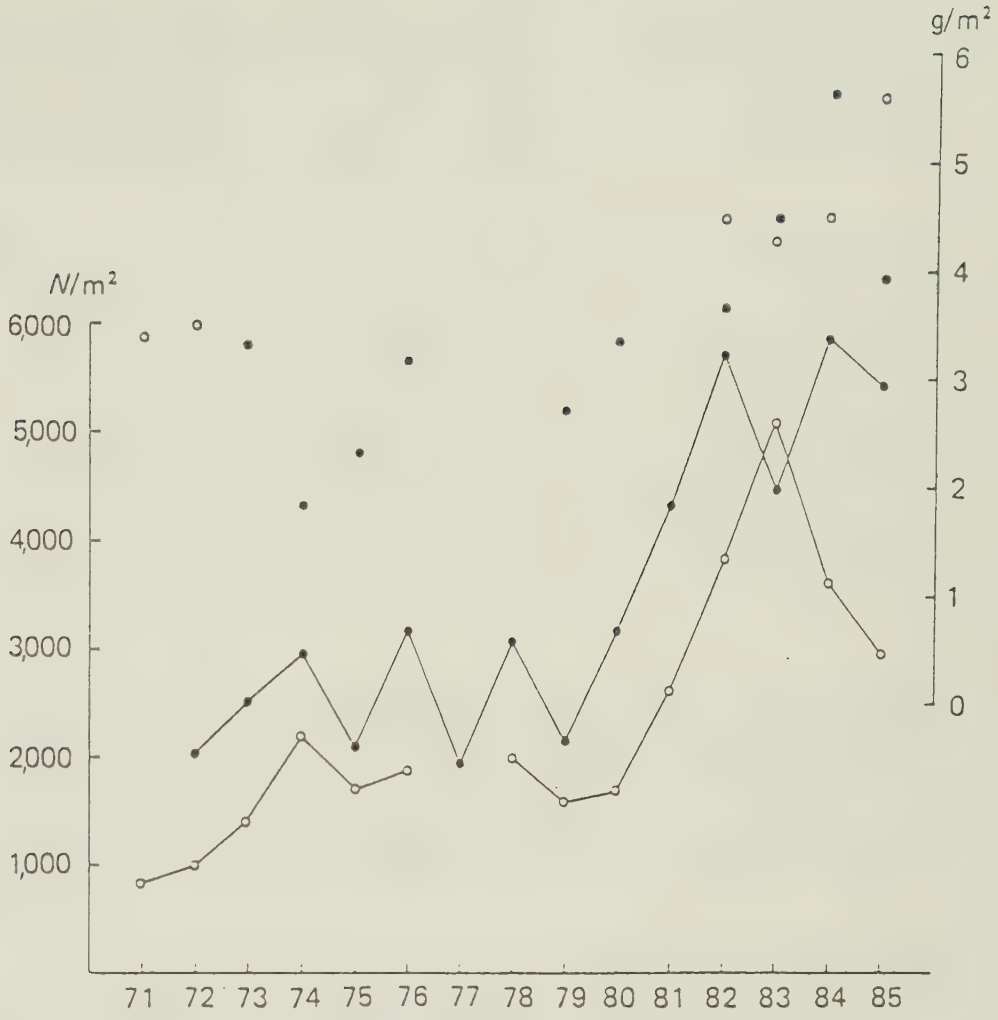
In terms of application of some of the newer techniques both to regular monitoring and to setting an EQS for an SDG the first candidate for inclusion will be a sediment toxicity test. This may make use of a single species or may follow the lines of the dredged spoil multi-species test. The standard would be set in terms of a reduction in survival of the test species in the sediment from the disposal ground compared with the survival in the reference sediment. The stations used for sampling may be at the licensed site boundary or may be set on transects through the ground. The approach of determining sediment quality criteria is currently being used elsewhere (Dickson, Maki and Brungs, 1987).

The next factor which might be applied is the water column toxicity. The usefulness of a water column toxicity test based on a single or series of samples taken at any one moment in time can be questioned. It is unknown whether samples are representative of the overall impact since the pattern of the dispersion plume will vary depending on hydrographical conditions. A better approach would be to identify an area outside which there should be no toxic effects due to the disposal. Then an annually or seasonally averaged dispersion pattern would be determined by use of a mathematical model and the dilution contour appropriate to the designated area identified. Based on the dilution contour, toxicity tests would be carried out on suitably prepared dilution series of the sludge.

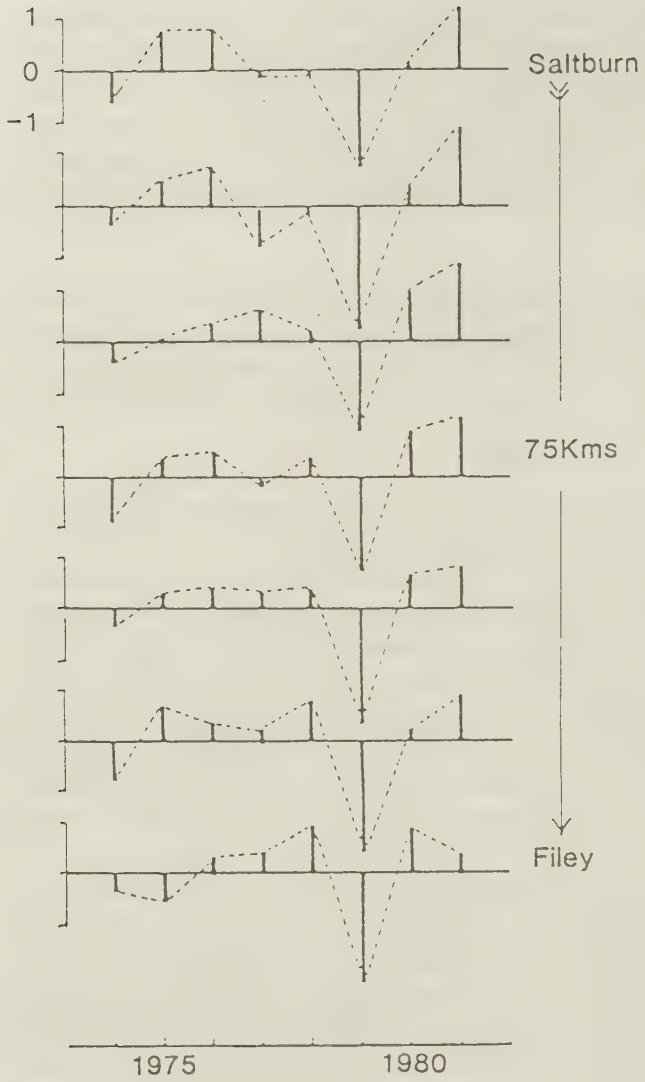
A final development using the techniques currently under evaluation would be the application of *in situ* physiological techniques, such as scope-for-growth (SFG) in marine mussels. The advantage of these methods is that the response integrates the effects of the pollutant over a number of days or weeks, thus overcoming the problem associated with "one-off" samples. With regard to setting an EQS, these techniques are still at an investigatory stage, and it is possible to suggest only that the EQS would be set in terms of a given percentage reduction in SFG; the degree of reduction cannot at the present time be identified.

A Hypothetical Standard for Permissible Quantitative Change in the Benthos at Sewage Sludge Disposal Sites

The aim is to determine change as a quantifiable deviation from the normal situation, thus avoiding exceeding the standard owing to natural temporal fluctuations (see Appendix Fig. 4 for examples of the latter). Hence the method relies on change relative to a "reference" site. The hypothetical standard is defined as follows: "Change at a sludge-affected site should not exceed $\pm x$ on a baseline value, sustained for a minimum of y annual sampling periods". The baseline value is set by calculating the % difference between treatment and reference measures at time zero.



Appendix Figure 4 a). Variation in the number of individual (N, joined), and the biomass (ash free dry weight), per square metre, at stations M1 (March, •) and P (January, o). These are at 55 and 80 m depth respectively off the Northumberland coast (from Buchanan and Moore, 1986).



Appendix Figure 4 b). Annual recruitment variation in ***Balanus balanoides*** at seven locations along 75 km of the north east coast of England. Note the high degree of similarity between all sites indicative of similar widely-operating factors. Data are expressed in terms of standard deviations astride each eight year mean. (From Commission of the European Communities, 1985.)

Whatever the chosen measure, initially the limits would almost certainly have to be arbitrarily set but would be aimed at maintaining the *status quo* within previously defined boundaries. In this hypothetical case, positive as well as negative change is considered to be potentially significant. This accords with what is known about the response of a variety of measures of benthic communities along gradients of organic enrichment.

On its own, the measure is unlikely to say much about the nature of change that may occur within these boundaries. As a result, a parallel series of qualitative standards must be set which would permit the input of expert interpretation. This might involve establishing a general or substratum-specific list of "pollution indicator" species. Such standards should be more open-ended in their formulation (but not woolly) relative to the quantitative standard (above), and should allow for an over-riding role of i) expert judgement, if necessary using a variety of measures of data structure, and ii) advances in scientific knowledge. This recognises that while the ultimate goal will be to eliminate subjectivity in assessing waste impacts on the marine biota, the present state of knowledge prevents this goal being achieved.

Advantages of hypothetical quantitative standard

The standard allows for the natural occurrence of synchronous temporal changes at "treatment" and "reference" sites. As a relative measure, it avoids the difficulty of assigning value judgements ("good" or "bad") to absolute values of the chosen measure, which may exhibit considerable natural variability from one locality to another. Trends may be monitored, allowing action to be taken in appropriate cases before the standard is exceeded.

The standard provides a degree of latitude in sampling/analytical practices between dump sites, eg difference in mesh size and difference in performance of sampler on consolidated/unconsolidated sediments. But this should not be taken to imply that quality control is unnecessary: clearly, once they are set, the procedures must be strictly adhered to for a particular dump site.

Requirements for effective implementation

A good knowledge of waste dispersion, zone(s) of impact and sediment heterogeneity at the sea bed is required to delineate "treatment" and "reference" sites. This may result in the sites being defined by single points or by larger areas containing a number of sampling stations.

Replication at a minimum of three samples per site will be necessary. The precise requirements will vary according to locality. For example, three may be adequate for uniform muds, but more will probably be required for sands or locally patchy environments. Sampling design must be statistically valid and expert advice must be sought before commencing the survey. Good re-location and consistent sampler performance will be necessary.

Environmentally similar conditions must prevail at treatment and reference sites. As far as possible like must be compared with like, since the occurrence of natural changes which are out of synchrony between sites could render the approach invalid. Sampling must be carried out at the same time of year.

Careful thought is required not only to establishing a statistically sound approach but also to empirical testing, preferably using existing long time series data sets which are known to exhibit trends in response to organic inputs.

Precautions

This hypothetical approach is presented simply as a measure of change and, without a good deal of empirical testing, could not be objectively linked to a sequence of events which would allow prediction of wider environmental effects. To achieve this, expert judgement would continue to be required. At present, such an approach could be applied only to soft substrata (typically ranging from sand to mud) which can be reliably sampled quantitatively by grab. Further work is required before a comparable approach could be proposed for the benthos of coarse/hard substrata, which cannot be reliably sampled by grab.

The baseline value, against which change will subsequently be measured, must be representative. Ideally, this should be determined from an average of at least three annual treatment and reference comparisons, at the same time of year and at constant input rates (see below). Note that pre-disposal data will be unavailable for most disposal sites currently in use. (This is unlikely to be the case for future areas).

A fixed value of permissible change applied to assemblages which differ in the chosen measure (eg diversity) from area to area will mean in practice, that greater unit change in values may occur at high diversity than low diversity sites before the standard is breached. It could be argued that this is intrinsically reasonable, since assemblages of naturally low diversity may be taken to indicate some degree of adaptation to "stress", and this often takes the form of dominance by relatively resilient species. Negative responses of such assemblages to waste inputs could, therefore, be interpreted as being more critical than those initially occurring within sensitive components of high diversity assemblages.

Other situations may arise, eg where treatment values are already maintained at a low level relative to reference values owing to anthropogenic effects. In such circumstances, the application of a stricter standard may be necessary.

The definition of a baseline could be problematical at new sites or those where significant alterations to the nature or quantity of wastes occur with time, since impacts on the benthos may take time to stabilise. For example, the response to a significant reduction in organic inputs at an over-enriched site could be an increase in diversity, while at a site exhibiting more benign effects a decline in diversity might be anticipated. This level of acceptable change will therefore have to be site-specific.

In some circumstances, establishing satisfactory treatment and reference sites to monitor sewage sludge disposal may be very difficult, eg in the case of other licensed waste inputs to a dump site or inputs from nearby disposal grounds, outfalls or rivers which may have the potential to influence one or both sites.

This potentially confounding influence of multiple inputs applies to a number of UK sludge disposal sites. Judicious selection of sites may go some way to resolving this problem, but in such environments the use of the term "reference" should not be taken to imply pristine conditions.

Although data from wide-scale spatial grid surveys would not necessarily lend themselves to the calculation of hypothetical standards in this context, it must be strongly emphasised that such surveys are essential for periodic checking of the spatial extent of impact, and hence the validity of treatment and reference sites.

The continuation of such surveys is essential to the effective implementation of the above hypothetical approach to standard-setting.

A comparison of treatment and reference sites may be adequate in cases where the system is well understood and stabilisation of impacts in response to many years of more or less constant waste inputs may be assumed. Where there is uncertainty as to waste impacts, eg regarding spatial localisation or stability of response, then it will be essential to introduce additional sites. If such an approach were to be implemented, then a rider must be added that any standards may be subject to change, in line with future improvements in understanding of the significance of ecosystem changes and in capabilities for detection of anthropogenic effects. It should also be noted that a parallel series of standards will almost certainly be required to quantify adequately change in different properties of community structure.

Conclusions

At present, change in the benthos cannot automatically be linked to effects on fisheries resources or human health. Even so, it would represent a target in the maintenance of local sea bed quality. It must be stressed that a good deal of empirical "fine-tuning" would be required before the use of any such standard could be formally applied.

While there are several reasons why sedentary benthos is an appropriate target for investigating local sea bed impact, consequential or independent effects on other components, eg pelagic or mobile benthic organisms, can rarely be predicted with certainty, given the present state of understanding of marine ecosystems. In this sense, the criteria of limited change in the benthos within a mixing zone and no change outside can be adopted as a precautionary measure.

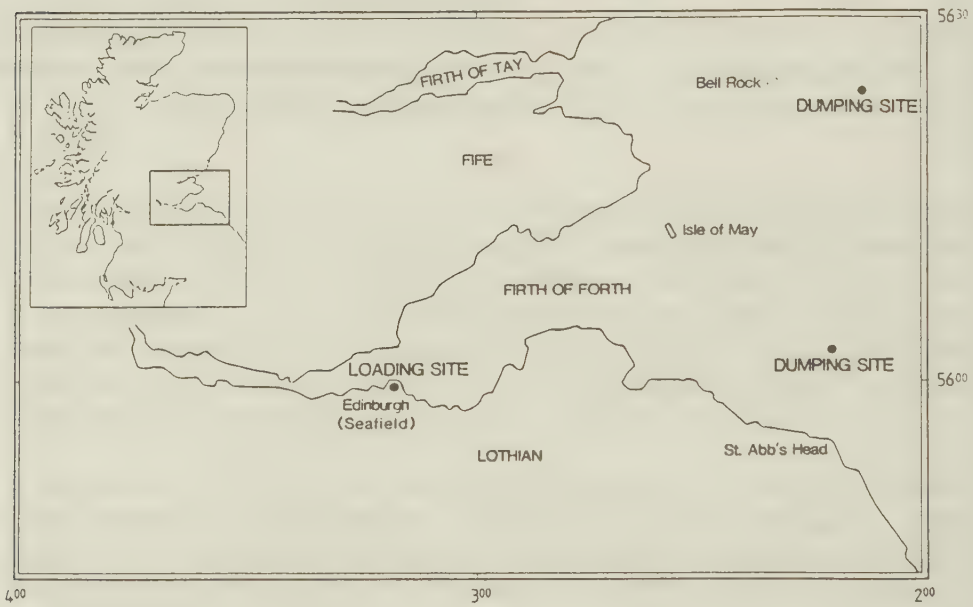
Appendix III
Sampling Strategies for Lothian and Strathclyde Region Disposal Sites

Introduction

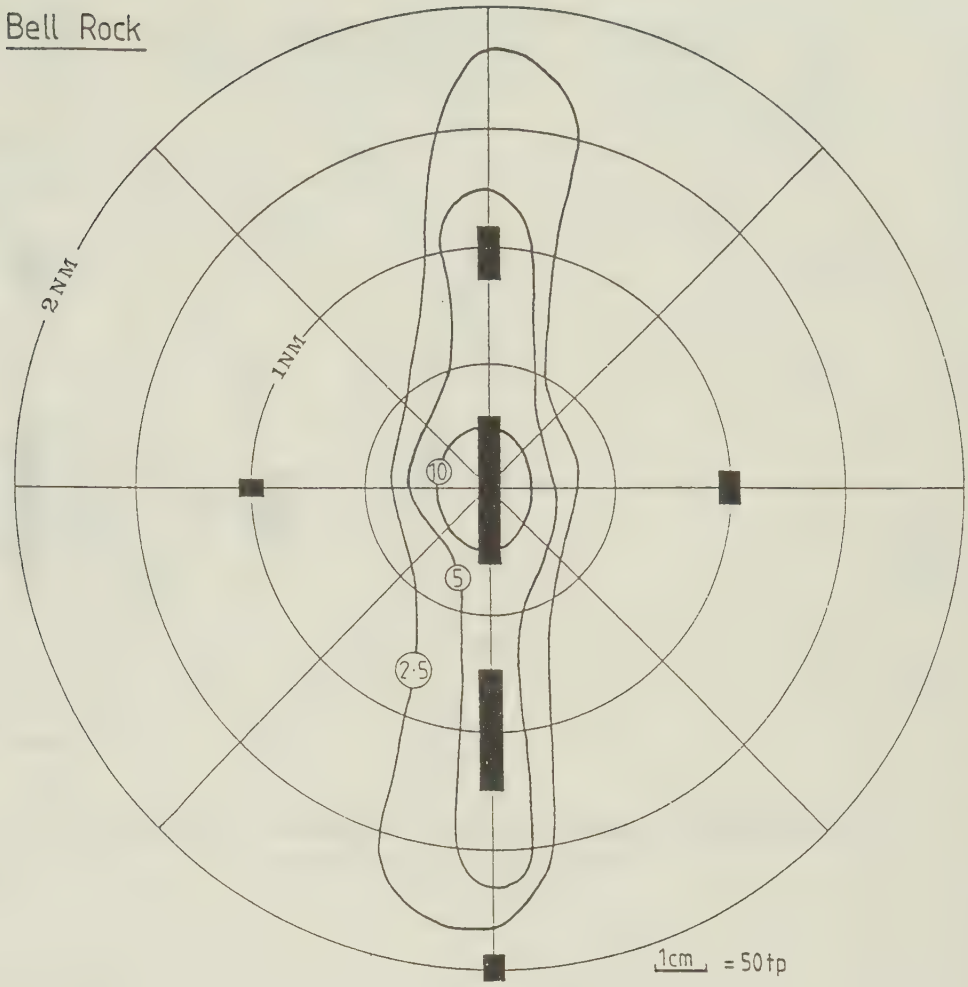
The Lothian and Strathclyde sewage sludge disposal sites represent the opposite ends of the spectrum of dispersive characteristics. Lothian Regional Council's grounds at Bell Rock and St Abb's Head have highly dispersing properties, while the Strathclyde Regional Council's site at Garroch Head is held up as the classical accumulating ground. The sampling strategies employed reflect this difference.

Bell Rock and St Abb's Head

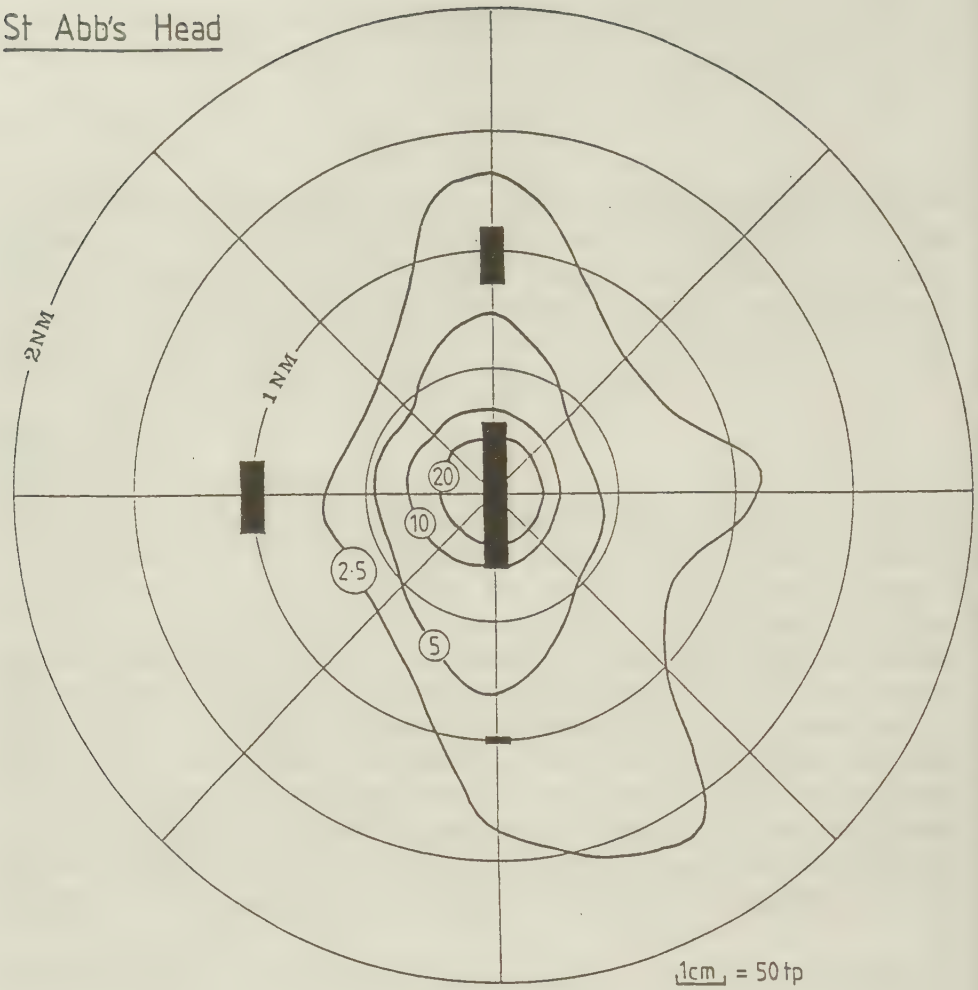
The two sites are used on a rotational basis and allow for site switching in cases of *force majeure*. Both these sites employ a similar sampling pattern based on stations arranged such that a series of transect lines intersect a series of concentric circles (Appendix Fig. 5). This gives a higher concentration of stations at the (licensed) centre of the disposal site and reduced concentrations in areas more remote from the likely site of impact. This strategy was devised before the dumping operation commenced. A sludge dispersion model was devised for both sites based on real hydrographic data and the dispersion contours for Bell Rock and St Abb's Head are shown in Appendix Figures 6 and 7 respectively. Superimposed on these diagrams are values for tomato pips found in the biological samples, and although the fit is not perfect there is reasonable agreement.



Appendix Figure 5. Location and station sampling pattern for Bell Rock and St Abb's Head sewage sludge disposal sites.



Appendix Figure 6. **Predicted dispersion pattern of sludge (contours) and observed distribution of "tomato pips" (tp) at Bell Rock sewage sludge disposal site.**



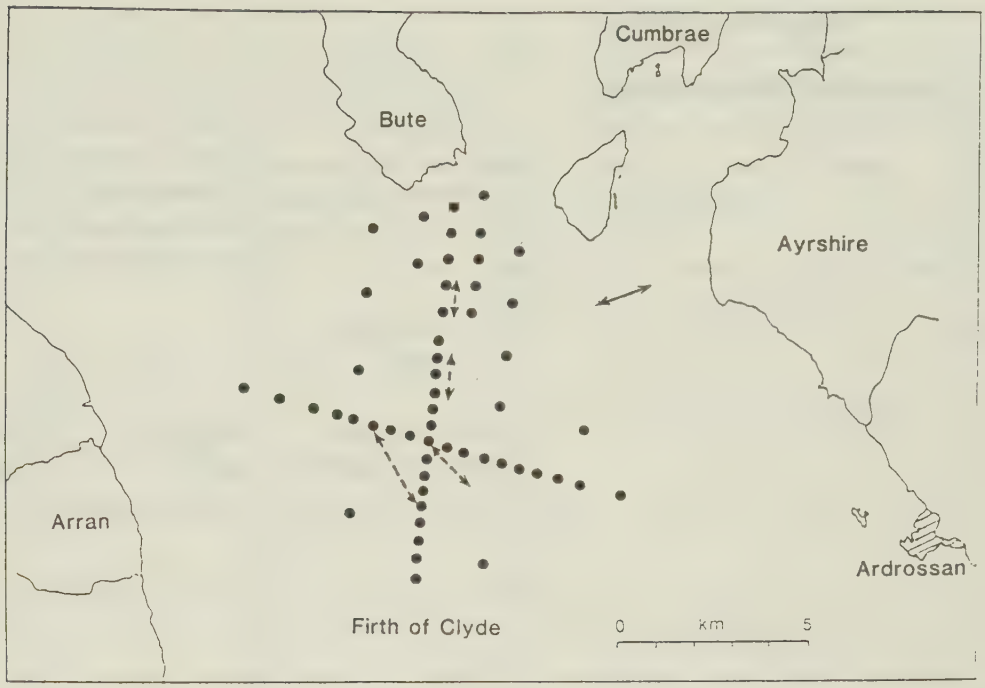
Appendix Figure 7. Predicted dispersion pattern of sludge (contours) and observed distribution of "tomato pips"(tp) at St Abb's Head sewage sludge disposal site.

The detailed sampling strategy is given in Appendix Table II. Benthos stations are outnumbered by those stations which need to be sampled for contaminants. A recent development has been the inclusion of a control station at both sites and both the NW-SE and NE-SW transects have been extended to give greater spatial coverage for contaminant detection.

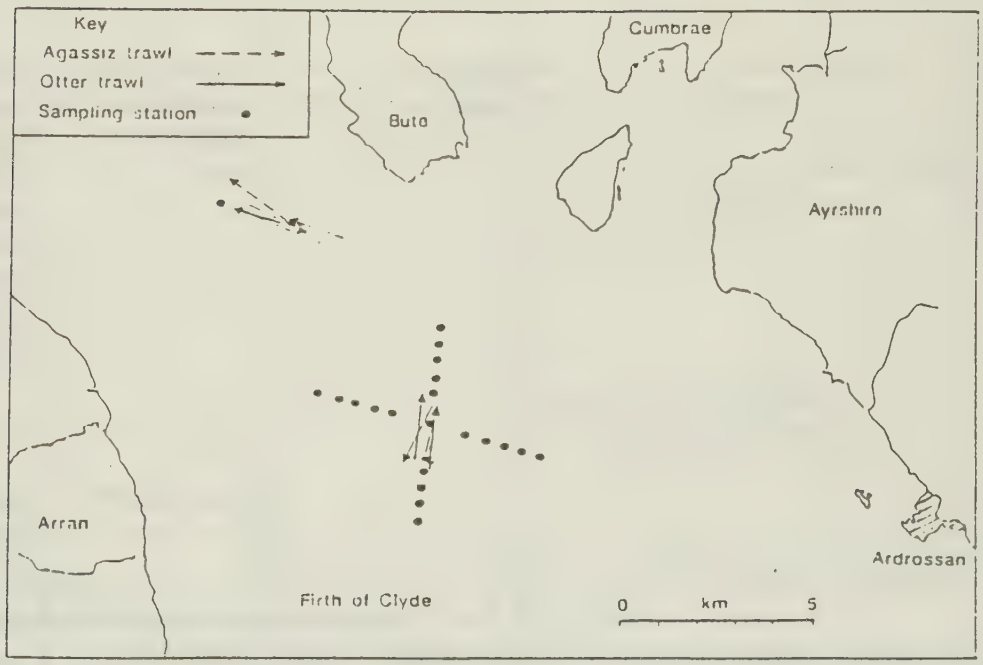
Garroch Head

When routine monitoring began at this site the survey strategy called for a grid system to cover the entire area of impact (Appendix Fig. 8). As time progressed this was gradually reduced to 22 stations as successive refinements were applied to the strategy (Appendix Fig. 9). As at the Lothian sites the number of contaminant stations outnumbers those sampled for benthos.

This strategy was devised to study an already impacted site and once the initial monitoring phase had been completed a more targeted effort could be applied in order to detect changes in the spatial area of impact.



Appendix Figure 8. Initial station sampling pattern at Garroch Head sewage sludge disposal site.



Appendix Figure 9. Refined station sampling pattern at Garroch Head sewage sludge disposal site.

Appendix Table II

Summary of monitoring

Sampling stations	Sampling requirements	Sample treatment
(i) St Abb's Head Control station (total one station)	One grab sample (total one grab)	Sub-sampled for the following:- physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm) organochlorines microbiology
	Otter trawl*	Preparation of species list Pathological examination Microbiology of gut contents
Station 13 (total one station)	Three grab samples (total three grabs)	Two grabs for macrobenthos and fruit pips One grab sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm) organochlorines microbiology
	Otter trawl*	Preparation of species list Pathological examination Microbiology of gut contents
Stations 1, 3, 9, 11, 15, 17, 23, 25, 27, 29 (total 10 stations)	Three grab samples (total 30 grabs)	Two grabs for macrobenthos and fruit pips One grab sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm) organochlorines

Appendix Table II (continued)

Sampling stations	Sampling requirements	Sample treatment
Stations 4, 5, 6, 7, 8, 12, 14, 18, 19, 20, 21, 22, 26, 28 (total 14 stations)	One grab sample (total 14 grabs)	Sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm)

* trawls to be confined to an area within 0.75 nautical miles radius of sampling station.

Total St Abb's Head sampling requirements:

26 sampling stations; 48 grab samples;
otter trawls (number dependent upon collection of sub-samples).

Sampling stations	Sampling requirements	Sample treatment
(ii) Bell Rock		
Control Station (total one station)	One grab sample (total one grab)	Sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm) organochlorines microbiology
	Otter trawl*	Preparation of species list Pathological examination Microbiology of gut contents
Station 13 (total one station)	Three grab samples (total three grabs)	Two grabs for macrobenthos and fruit pips One grab sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm) organochlorines microbiology

Appendix Table II (continued)

Sampling stations	Sampling requirements	Sample treatment
	Otter trawl*	Preparation of species list Pathological examination Microbiology of gut contents
Stations 1, 3, 9, 11, 15, 17, 23, 25 (total 8 stations)	Three grab samples (total 24 grabs)	Two grabs for macrobenthos and fruit pips One grab sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm) organochlorines microbiology
Stations 4, 5, 6, 7, 8, 12, 14, 18, 19, 20, 21, 22, 26, 27, 28, 29 (total 16 stations)	One grab sample (total 16 grabs)	Sub-sampled for the following: physical appearance particle size carbon and nitrogen (incl <63 µm) heavy metals (incl <63 µm)

*trawls to be confined to an area within 0.75 nautical miles radius of sampling station.

Total Bell Rock sampling requirements:

26 sampling stations; 44 grab samples;
otter trawls (number dependent upon collection of sub-samples).

Appendix IV

A Bibliography of Taxonomic References

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Unpublished material cited above may be obtained by contacting the Forth River Purification Board, Heriot-Watt Research Park, Avenue North, Riccarton, Edinburgh EH14 4AP.

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